

The Role of Monkey Prefrontal Cortex in Encoding Multiple Items in Working Memory

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University of Pittsburgh, 2013

As we interact with our environment we are confronted with a constant barrage of sensory information that has to be stored and manipulated, often within a very short period of time. Working memory is the mechanism that supports the active maintenance and manipulation of information. The ability to hold and use information in memory is fundamental for cognition. Many complex behaviors require that we store several pieces of information in working memory. The requirement to remember several things at once is ubiquitous to most of our day to day interactions, yet very little is known about its neural basis.

To explore the neural mechanisms underlying multi-item working memory we recorded activity of neurons in the prefrontal cortex (PFC) of two monkeys while they performed a Sternberg working memory task. On each trial, three samples were presented in succession, each followed by a delay. The monkeys were required to hold these three samples in memory and recall one of them at the end of the trial. The monkeys' performance decreased when working memory load was high. The monkeys' also exhibited a recency effect where performance was better when the item to be recalled was the most recent.

PFC neurons were selective for the identity of the sample during the delay and selectivity tended to be the same regardless of the ordinal position of the sample. However, the strongest

determinant of activity during each delay-period was the identity of the immediately preceding sample. The degradation of information about earlier samples stood in striking contrast to the monkeys' intact ability to perform the task.

We found that the mean population firing rate and power in the gamma frequency band of the LFP increased as samples were presented successively. However, these effects were not related solely to memory load, because a similar pattern was present under the control condition in which the same sample was presented three times.

From the results of these experiments we conclude that although monkeys were capable of performing a task requiring the memory for multiple items, delay-period activity in prefrontal neurons cannot easily explain the monkey's working memory performance.

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PREFACE

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1.0 GENERAL INTRODUCTION

The term working memory refers to the cognitive function which enables us to keep information on-line so that it may be manipulated and used to make decisions. Working memory is necessary to maintain focus in the face of distractions and allows us to keep an updated representation of the world around us. It is known that working memory capacity increases as we progress from infancy to adulthood. Furthermore, many view that increases in working memory capacity over a much longer period of time have been central to the evolution of advanced human cognition (Balter, 2010). This enhanced capacity has given us the ability to reason, problem solve, plan etc., and has likely been crucial for the development of language and mathematics.

Working memory, thought largely to be supported by prefrontal cortex (PFC), is one of our most crucial cognitive abilities, essential for countless daily tasks. There have been many neurophysiological studies that examine how single items are maintained in the activity of prefrontal neurons. However little is known about the encoding of multiple items. This is an important question because complex behaviors require that many items be held in memory at one time.

It is well established that our ability to remember multiple items is supported by a resource limited in capacity. Many theories exist that attempt to explain the structure of these resources. There has been little effort devoted to defining what “resources” means in neural

terms. Understanding the biological underpinnings of working memory requires clarification of how multiple items are stored and how resources are allocated by prefrontal cortex.

1.1 THEORY OF WORKING MEMORY

The concept of working memory refers to the theoretical construct which allows individuals to hold information in mind for brief periods of time (Baddeley and Hitch, 1974). Working memory, though operating over a timescale of mere seconds, is central to comprehension, planning, and decision making processes. Holding information in this temporary store provides us with a mechanism to integrate and manipulate information from our surroundings, memories, or internal state so that we may interact and adapt to our ever-changing environment.

A well-established characteristic of working memory is its limited capacity. Miller (1956) was among the first to demonstrate working memory capacity is limited to approximately 7 ± 2 items. Though it is now well-known that working memory capacity is limited, it is less known what the nature of these limits are. Luck and Vogel (1997) proposed that working memory stores a fixed number of items (3-4) with a fixed resolution. Others propose that the pool of working memory resources can be allocated flexibly with varying resolution that is dependent on working memory load (Frick, 1988). Since the studies by Frick, others have attempted to quantify and characterize the structure of these limitations with varied results (Zhang and Luck, 2008; Bays and Husain, 2008).

1.2 ANATOMY OF WORKING MEMORY

Delayed-response tasks have often been used as a test of working memory. In these tasks, subjects are required to respond on the basis of stored internal representations maintained throughout a delay-period without external sensory information. PFC lesions produce profound performance deficits on delayed response tasks (Jacobsen, 1935). The delay makes these tasks strongly dependent on working memory function. Since then, many neurophysiological studies have demonstrated that neurons in PFC exhibit sustained activity during the delay-period which requires active maintenance of information to respond correctly (Funahashi et al, 1989; Funahashi et al, 1993; Fuster and Alexander, 1971; Miller et al, 1996). This delay-period activity is thought to be the neural correlate of maintaining information “on-line”, i.e. working memory.

Delay-period activity of PFC neurons has been described as the “memory trace” of a recently experienced stimulus. Neuronal activity is often selective for specific stimulus features during the delay-period. It is not clear whether neuron selectivity is constrained to a particular domain (e.g. location versus object); that is, are features processed separately by different populations of neurons or is this information integrated by PFC neurons? Some studies have reported that object and spatial information remain segregated in lateral PFC. Spatial information is processed dorsally and object information is processed ventrally (for review, see Levy and Goldman-Rakic, 2000). However, studies reporting these findings may have artificially produced this apparent segregation by examining “what” and “where” with separate tasks. Feature and spatial information does not remain segregated in PFC when engaged in a task that requires information to be integrated across these two domains. Rao and Miller (1997) reported that in tasks requiring that object and location information be used together within a trial, populations that encode the conjunction of these features emerge. These populations are not topographically

segregated but are equally distributed throughout dorso- and ventrolateral PFC. It appears a subset of PFC neurons integrate information across domains, a process necessary for the guidance of action towards objects.

1.3 GAMMA BAND SYNCHRONY AND WORKING MEMORY LOAD

Populations of neurons synchronize their activity in a variety of frequency ranges. There is now a large body of evidence implicating high frequency gamma oscillations (~40 Hz and higher) as a critical mechanism for binding sensory features into a coherent representation (for review, Ribary, 2005; Bertrand and Tallon-Baudry, 2000). Recent studies have reported that synchronization of cortical neuronal activity in the gamma band is not only associated with various types of motor and sensory processing, but also extends to higher cognitive processes like working memory (Howard et al., 2003; Tallon-Baudry et al., 1998; Tallon-Baudry et al., 1999). Working memory allows us to keep items in mind after they are no longer available to us as physical stimuli. Keeping items in mind requires that representations of these items be sustained until they are used for subsequent action. The amount of information that must be held in mind is referred to as working memory load. Providing evidence for the involvement of gamma synchronization in the maintenance of multiple-items in memory, Howard et al. (2003) reported that gamma oscillations increased linearly with increasing working memory load. Specifically, it has been suggested that gamma oscillations support the organization and temporal segmentation of multiple items in working memory (Lisman and Idiart, 1995; Luck and Vogel, 1997; Jensen and Lisman, 1998).

1.4 MULTI-ITEM WORKING MEMORY

There has been a considerable amount of effort dedicated to characterizing how PFC neurons represent information about single items maintained within working memory. Yet, little is known about how multiple items in working memory are represented. This is an important question. Outside the laboratory, it is more common to hold many items in memory to effectively interact with our environment. The necessity for multi-item working memory can be extended over a variety of complex cognitive processes. For example, holding numbers in memory to perform arithmetic operations, learning associations, or remembering a phone number we just heard. Based on what we know from the extensive single item literature, one possibility is that separate groups of neurons in PFC maintain the memory of each item. An alternative possibility is that neurons in PFC are capable of simultaneously representing sets of items and this representation is distributed over a single population of neurons. Though there have been many theories proposed as to how multiple item memories may be stored, only three neurophysiological studies have been published which address this question directly. Inoue and Mikami (2006) trained monkeys on the serial probe reproduction task. Monkeys were required to view two objects sequentially interleaved with a 1 second delay. Following the second object delay-period, monkeys were presented with a cue that instructed the animal to select either the first or second item of the set. Following the cue, the monkey was presented with an array of three items and was required to select the item from the set as instructed by the preceding cue. They found that task related neurons were localized to ventrolateral PFC. Separate populations of neurons exhibited selectivity for epoch, objects, temporal order, and sometimes combinations. Object information was not carried across epochs of the trial by single neurons. The authors proposed

that in PFC information flows between neurons selective for adjacent processing stages including encoding, maintenance, and retrieval during multi-item working memory tasks.

Warden and Miller (2007) also addressed this question of multi-item working memory by using a simpler match/non-match to sample task. In their version, the monkey was required to identify whether a sequence of complex objects matched or did not match a previously viewed sequence. Both the sample and test sequences consisted of two complex objects interleaved by a 1 second delay. They compared the first delay (only 1 item has been presented) to the second delay (both items had been presented). The prediction was that if neurons encode the identity of multiple items, then this should be reflected in the second delay-period. They discovered that the majority of PFC neurons encoded the identity of both items, though not in a straightforward way. Activity related to the sequence of the two items could not be predicted by the neuron's single item activity.

Warden and Miller also examined how selectivity from the first delay-period related to that during the final delay. Logically it is possible that neurons selective for an item maintain that information across delay-periods, though the representation may become weaker as the trial persists. On the contrary, they found that although some neurons represented the first item of a sequence during the final delay-period, the selectivity expressed by the neuron can not be predicted from that which is expressed during the first delay. It is a paradoxical finding that the representation of the first item during the first delay is not concordant with its representation during the final delay. It is important to determine if the paradoxical finding reported by Warden and Miller can be replicated. The authors reported that the objects used in their experiments did not optimally drive PFC neurons. Neurons did not show strong selectivity to one item as evidenced by very broad tuning for objects. It is difficult to discuss selectivity for an item when

using stimuli that do not optimally drive PFC neurons. It is important to know if the same paradoxical findings persist when using items that optimally drive PFC neurons.

Both studies found that when remembering a sequence of items, information about the first item in the sequence is represented in the final delay-period activity. When comparing the strengths of item representation during the final delay-period, Warden and Miller reported that recent items are strongly represented during the final delay, and earlier items have weak representation. This is in direct contrast to what was reported by Inoue and Mikami, who found that in the majority of neurons with selective final delay-period activity, the signal strongly represented the identity of the first item. This discrepancy may be accounted for by the differences in the read-out of information maintained in memory; one relying on recall the other on recognition. Warden and Miller (2010) examined this possibility and found that read-out of the memory affected selectivity in the two object delay-period. Using a task that differed only in the response type required (recall or recognition); they found that when monkeys were required to merely recognize a match or non-match, selectivity was largely for the item most recently seen. However, when the monkeys were required to explicitly recall an item maintained in memory (by reproducing the item set sequence) the selectivity during the object delay-period was dominated by the first item in the set. This could not be attributed to separate populations of neurons specific for recognition vs. recall as they found this pattern of activity within a single neuron. This activity may reflect a prospective code required to reproduce the sequence of the item set which was not required in the match to sample task.

These studies began the process of interrogating the mechanisms of multi-item working memory. However their conclusions are by no means complete. It remains to be determined if item representation degrades as new items are added to memory, and if so how does this affect

performance. To do this one would have to use a different task design than used in the previous multi-item working memory studies. One modification would be to remove the sequence memory component and use an approach similar to the Sternberg paradigm. Memory for sequences requires that all items in the set be remembered in order. Employing this design prohibits one from asking if memory for earlier items is worse and how this relates to the underlying neural activity. Another modification would be to add more items so that questions regarding working memory load could be answered.

1.5 CUED RECALL STERNBERG TASK

To get a clearer understanding of how multiple items are held in working memory, we monitored single-neuron activity and local field potentials (LFPs) in the PFC of two monkeys trained to perform a Sternberg task. This task eliminates the necessity to remember sequences. This allowed us to ask questions regarding increases in working memory load as well as how the representation of an item is degraded as items are added to memory. In one version of the task, the items were locations and, in the other, they were colors. We used simpler stimuli with an easily described feature space and whose representation in PFC is much better understood. On each trial, three samples were presented in succession, each followed by a delay-period. Then three probes were presented simultaneously. One probe matched a preceding sample. The monkey was rewarded for making a saccade directly to the match probe.

1.6 EXPERIMENTAL AIMS

There are two primary goals for these experiments. (1) Determine the code used to represent multiple items in PFC. (2) Determine whether this code becomes less efficient with larger item sets. To achieve these goals I have 5 specific aims:

Aim 1: Demonstrate that monkeys can perform the cued-recall Sternberg Task

Few experiments have examined monkey performance on the Sternberg working memory task. The goal of this first aim was to demonstrate that monkeys can perform a cued recall version of the Sternberg working memory task and determine if performance is comparable to that which has been observed in humans.

Aim 2: Determine if there is a correspondence of information across delay-periods within a trial during a multi-item working memory task.

Results from previous studies examining the maintenance of information during multi-item working memory tasks are mixed and difficult to interpret. The goal of this aim was to determine if there was a correspondence of selectivity across intervening delay-periods in the context of the Sternberg task.

Aim 3: Determine the code for the representation of multiple items in the population activity of PFC as a whole

PFC neurons may encode multiple items in two obvious ways (1) combining sets of neurons that represent individual items (main effect code) or by new sets that are selective for conjunctions of

items independent of individual item selectivity (conjunction code). Our goal was to determine which code is used by the neuronal population in PFC using population decoding methods.

Aim 4: Behavioral correspondence with neural observations.

Using the same set of working memory tasks, we determined whether there was a correlation between strength of the neuronal representation of stimulus identity and two measures of performance: accuracy and reaction time.

Aim 5: Determine if gamma synchronization increases with increasing working memory load.

It has been demonstrated in humans that PFC gamma synchrony increases with higher working memory load (Howard et al., 2003). This phenomenon has not yet been explored in monkeys. Our goal was to determine if this is also true in monkeys. We recorded local field potentials in monkeys performing the same tasks used in Aim 1 and asked: (1) is gamma synchronization present during the delay-period? (2) does it increase as items to be remembered are added within the trial?

2.0 PERFORMANCE ON CUED RECALL STERNBERG TASK IN MONKEYS

2.1 INTRODUCTION

The necessity to keep several items in memory at one time is something that we are confronted with daily. The number of items as well as the order in which they are presented affects how quickly and accurately these items can be recalled. When subjects are instructed to maintain several items in memory their ability to remember items at the beginning (primacy) and end of the list (recency) is better than for those that fall in the middle of the sequence resulting in a U-shaped serial position function (Murdock, 1962). Additionally, it has been demonstrated that as the item list size increases subjects are slower to respond regardless of whether the response is positive (“match”) or negative (“non-match”) (Sternberg, 1966). This phenomenon is thought to reflect a memory scanning process for item retrieval.

Serial probe recognition (SPR) tasks (e.g. Sternberg task) have been used classically to examine working memory properties in humans. A variety of manipulations have been applied to this task over the years to provide us with a richer understanding of multiple item working memory. It has been demonstrated that animals (pigeons and macaques) perform similarly to humans on SPR tasks using lists as long as twenty items (Sands and Wright, 1980).

Manipulations of the timing between the end of the item sequence and probe have been found to change the shape of the serial position function in both humans and monkeys (Wright et al, 1985). Also, humans and monkeys have been shown to be equally sensitive to increases in overlap between item features within a set. As items within a set become more similar both humans and monkeys respond less accurately. This is thought to be a result of proactive interference between set items (Sands and Wright, 1980). The consistency in a multitude of observations across species using the SPR task has lead to the conclusion that similar mechanisms for memory retrieval are at play in both humans and monkeys.

Although it has been demonstrated that monkeys are capable of performing SPR tasks, the majority of neurophysiological studies investigating working memory rely largely on simpler working memory tasks using only one or two items to be remembered. This sort of task design does not allow us to examine primacy and recency effects nor does it allow us to ask questions regarding working memory load and performance. Additionally, these tasks are often confounded by the requirement to remember item sequences further complicating interpretations. To address these issues, we designed cued recall versions of the Sternberg working memory task where monkeys were required to remember a set of sequentially presented items. The Sternberg task is a classic working memory paradigm used primarily in humans. This task probes working memory without the requirement for sequence memory. This task allowed us to manipulate working memory load by varying the number of items to be remembered in a trial from either one or three. Monkeys were trained on cued recall versions of the Sternberg working memory task. Requiring responses based on recall rather than recognition (match/non-match) responses made the task more difficult for the monkey to perform thereby producing larger error rates. This allowed us to examine both response accuracy and reaction time.

There were two different versions of the task: one using color stimuli the other using locations. Color and location were chosen because these features are basic and can be described in a straightforward manner within a well-defined feature space. This is in contrast to previous studies which employed complex objects (Inoue and Mikami, 2006; Warden and Miller, 2007 and 2010). Another advantage to using these stimulus sets is that neurons in prefrontal cortex (PFC) have been shown to be selective for both color (Fuster et al., 1982; Inoue and Mikami, 2006; Quintana and Fuster, 1999) and location (Funahashi et al., 1989). This will be a useful task feature as we will go on to examine neural correlates of the working memory trace for these items described in subsequent chapters.

2.2 METHODS

2.2.1 Subjects

Subjects were two adult male rhesus monkeys (*Maccaca mulatta*) weighing approximately 8 and 9 kilograms at the time of the experiments. Daily intake of fluids was regulated in order to maintain motivation to perform the task. During the testing, monkeys sat head-fixed in a primate chair facing a 17 inch LCD computer monitor placed 58 cm away. Computers running NIMH Cortex (provided by Dr. Robert Desimone) served as the experimental control system. Eye position was monitored using scleral search coils (Judge et al., 1980) with a Riverbend field driver and signal processing filter (Riverbend Technologies Inc.). The outputs of the search-coil

were led to the computer via an A/D converter and monitored online through NIMH Cortex. The computer sampled and stored horizontal and vertical readings at a rate of 1 kHz. All procedures and experiments were conducted under the supervision of the University of Pittsburgh Institutional Animal Care and Use Committee and complied with the guidelines set forth in the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

2.2.2 Visual stimuli and behavioral task

The monkeys were trained to perform the Sternberg cued-recall task. Monkey 1 was trained on two different versions of the task; one using color stimuli the other using spatial stimuli. Training occurred over a period of approximately one year for each monkey. Within a year Monkey 2 was able to adequately learn only the spatial version of the task. The sample set for the color version of the task consisted of colored circles (1.3° visual angle in diameter) which could either be red, green, blue, yellow, magenta, or cyan. In the location version of the task the stimulus set consisted of white circles (1.3° visual angle in diameter) which could appear at one of six locations on a hexagonal array. Points on the array were located at an eccentricity of 5° . The array was centered on fixation at an orientation such that two of the points were on the horizontal meridian. The two tasks involved equivalent events with the exceptions that in the spatial task a grey hexagonal array appeared at the outset of the trial.

The trial began with the monkey fixating a grey fixation spot (0.3° visual angle) within a 3° window. Throughout the entire trial up to the time of the saccadic response, the monkey was required to maintain gaze within a 3° window centered on the fixations spot. Once fixation was maintained for 200 ms three different samples (colors/locations) were presented sequentially. Each sample appeared for 200 ms, followed by a 400 ms delay-period. Following the final

delay, the monkey was presented with an array consisting of three items: one match probe and two non-match probes. The match probe corresponded to one of the three items presented in the immediately preceding sequence. The monkey was required to make a saccade to the location occupied by the match probe. Once the monkey made a saccade to the probe, the distractors disappeared leaving only the probe on the screen. This served as feedback indicating a correct response. After fixating the feedback stimulus for 400-550 ms, the monkey was rewarded (0.1 cc liquid reward). The sequence of events in each task is shown in Figure 1. The monkeys were also trained on control conditions. Events under the control condition were identical to those under the experimental condition that the *same* sample was presented three times. The control task allowed us to examine the effects of working memory load on performance.

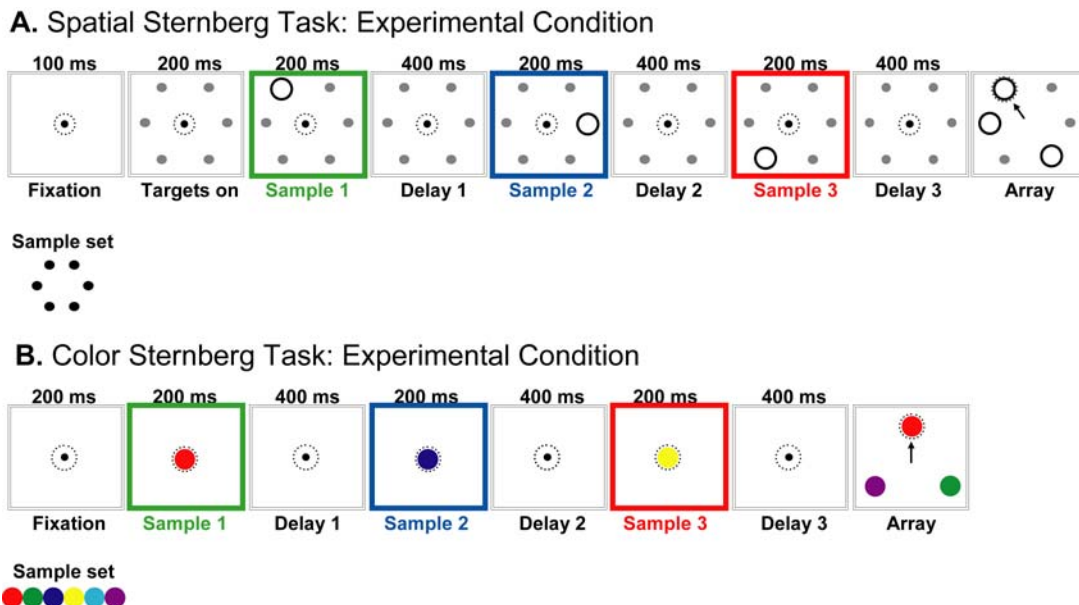


Figure 1. Sequence of events for the cued recall Sternberg Task

Dotted circle indicates monkey's eye position throughout the trial. Control condition events are identical with the exception that the same stimulus is repeated three times. Colored boxes were drawn to simply to illustrate the 3 epochs of the trial and were not presented to the monkey during testing.

In each session of a given task, the monkey was required to perform correctly four trials conforming to each of 36 conditions. The conditions represented all possible combinations of sample 1 identity (six possibilities) and sample 2 identity (six possibilities). If sample 1 and 2 were different, then sample 3 was chosen at random from the remaining four items. If samples 1 and 2 were the same, then sample 3 was chosen to match them. This design is summarized in Figure 2. The match probe and the two non-match probes in the final array were chosen randomly. In consequence of this design, 83.3 % of the trials were of the experimental condition, and 16.6 % of the trials were of the control condition. One session consisted of 144 correct trials. The order in which the conditions were imposed was random except for the requirement that each block of 36 successfully completed trials must consist of one trial conforming to each condition. Monkeys performed one session of each task on average per day.

A.		Sample 2					
		R	G	B	Y	M	C
Sample 1	R	4	4	4	4	4	4
	G	4	4	4	4	4	4
	B	4	4	4	4	4	4
	Y	4	4	4	4	4	4
	M	4	4	4	4	4	4
	C	4	4	4	4	4	4

B.		Sample 2					
		UR	R	DR	UL	L	DL
Sample 1	UR	4	4	4	4	4	4
	R	4	4	4	4	4	4
	DR	4	4	4	4	4	4
	UL	4	4	4	4	4	4
	L	4	4	4	4	4	4
	DL	4	4	4	4	4	4

Figure 2. Counterbalance procedure for Sample 1 and Sample 2.

The sample set for each task, A. Color, B. Spatial, consists of six items (color or location). The identity of the first sample is given on the vertical axis; the identity of the second sample is given on the horizontal axis of the diagram. The number within each box indicates the total number of trials of that particular sample 1/sample 2 combination. We did not counterbalance for the third sample. The grey boxes indicate the control trials where the same sample was repeated three times. All sample 1/sample 2 combinations were repeated 4 times to give a total of 144 trials.

2.2.3 Data Analysis

Analysis was confined to trials in which the monkey made a saccade to one of the three probes and maintained fixation on it until the end of the trial. All analyses were based on computing the mean of a measure for each session and then considering the distribution of the means across sessions. The measures of interest were percent correct and reaction time on correct trials.

Working memory load

We examined the effects of working memory load by comparing performance on experimental trials (where the load was high: three items) with performance on control trials (where the load was low: one item). For each monkey and each condition we computed the session mean reaction time and percent correct for the low and high working memory load conditions. We then normalized by subtracting from the high-load and low-load measures the mean of the two. We then examined the effect of working memory load using a two-factor ANOVA (Factor 1: high or low working memory load; Factor 2: match probe identity which had six levels) on the normalized mean reaction times and accuracies per session which served as the dependent variables.

Primacy and recency effects

Primacy and recency effects can only be observed on trials where the sample set consisted of three different colors or locations; therefore control trials were excluded for this analysis. We examined primacy and recency effects by comparing performance on trials where the probe matched either the first, second, or third sample in the sequence. For each monkey and each task we computed the session mean reaction time and percent correct for three conditions: match probe corresponded to sample 1, match probe corresponded to sample two, and match probe corresponded to sample 3. Again, we normalized by subtracting from these three measures the mean of the three. We examined the effects of position within the sequence on performance using a two-factor ANOVA (Factor 1: sequence position of match probe which had 3 levels; Factor 2: match probe identity which had six levels) on the normalized mean reaction times and accuracies per session which served as the dependent variables. We computed post-hoc analyses

of variance where we compared all possible serial positions of the probe stimulus: Factor 1 was the sequence position and had two levels: (1) sample one versus sample two (2) sample one versus sample three (3) sample two versus sample three. Factor 2 was the probe identity which had six levels corresponding to the color or location of the sample. Reaction times and accuracies served as the dependent variables.

2.3 RESULTS

Performance under the three-item conditions was significantly above chance (33.3%) for both monkeys. Monkey 1 performed at 86% correct on the spatial and 69 % correct on the color version of the task. On correct trials, Monkey 1 had a mean reaction time of 202 ms on the spatial and 250 ms on the color version. Monkey 2 performed at 70 % correct on the spatial task and had a mean reaction time of 252 ms on correct trials.

Both monkeys exhibited significant main effects of sample identity indicating a bias to respond more quickly and accurately to certain stimulus types. In the spatial task both monkeys were somewhat more accurately and quickly when the probe appeared on the left half of the screen. In the color task, Monkey 1 responded more quickly and accurately when the probe stimulus was either red or cyan.

2.3.1 Working memory load

A significant main effect of working memory load (low versus high) in both monkeys indicated that it was indeed more difficult to remember one item versus three. The monkeys were significantly less accurate on the high versus low working memory load condition (% Correct Low – % Correct High for M1 spatial: 25%; M2 spatial: 15%; M1 color: 19%, 2-way ANOVA main effect of load $p < 0.0001$). Monkeys were also significantly slower to respond when they had to maintain more items in memory (RT High – RT low for M1 spatial: 46 ms; M2 spatial: 20 ms; M1 color: 19 ms, 2-way ANOVA main effect of load $p < 0.0001$) (Figure 3. left panels).

2.3.2 Primacy and recency effects

A main effect of the match probe sequence position (whether it matched the first, second, or third sample) revealed that the position the probe occupied within the sequence, also had a significant effect on reaction time (M1 spatial: $p = 7.2 \times 10^{-7}$, M1 color: $p = 4.3 \times 10^{-5}$, M2 spatial: 1.7×10^{-14}) and accuracy (M1 spatial: $p = 1.2 \times 10^{-9}$, M1 color: $p = 3.8 \times 10^{-12}$, M2 spatial: 4.9×10^{-12}) (Figure 3. right panels). Post-hoc analyses revealed that the monkeys were significantly less accurate in selecting the probe when the probe matched the first item in the sequence compared to when it matched the third, more recently seen sample (% Correct probe matched 1st item – % Correct probe matched 3rd item for M1 spatial: 8%, $p = 1.9 \times 10^{-9}$; M2 spatial: 9%, $p = 2.1 \times 10^{-14}$; M1 color: 8%, $p = 2.9 \times 10^{-6}$). The monkeys were also significantly slower (RT probe matched 1st item – RT probe matched 3rd item for M1 spatial: 5 ms, $p = 4.1 \times 10^{-7}$; M2 spatial: 13 ms, $p = 8.7 \times 10^{-15}$; M1 color: 5 ms, $p = 1.4 \times 10^{-5}$).

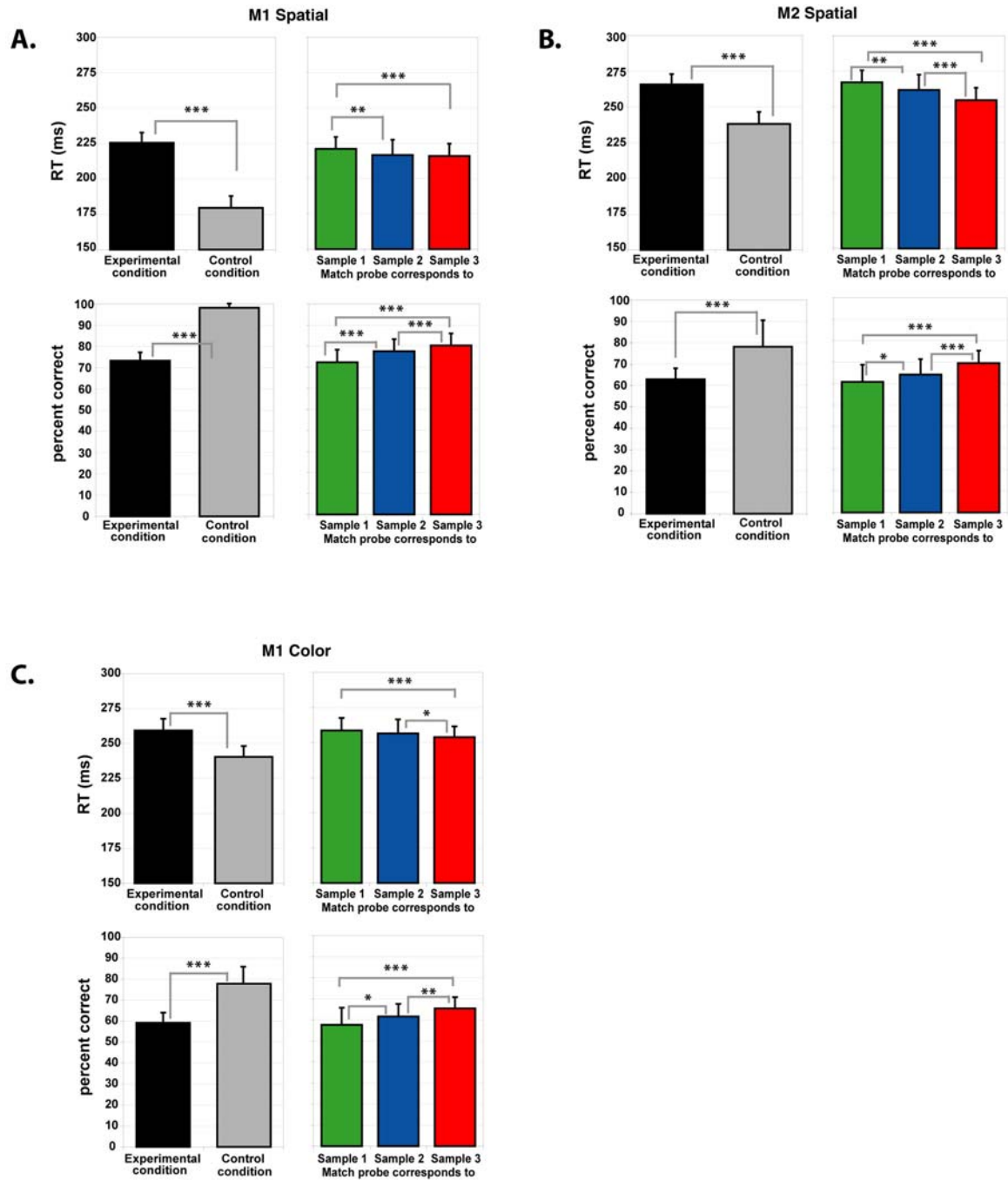


Figure 3. Behavioral performance on the Sternberg task.

Top: reaction time, Bottom: accuracy. Left (black and grey panels): experimental vs. control comparisons, Right (colored panels): effects of serial position of probe. Astrisks indicate significant main-effects from 2-factor ANOVA: *** ($p < 0.00001$), ** ($p < 0.0005$), * ($p < 0.05$). A-B detail results from the spatial version of the task, C. details the results from the color task (tested only in monkey 1).

2.4 DISCUSSION

Working memory in monkeys has been most commonly studied using various versions of delayed response or delayed match to sample tasks. After a brief cue presentation, these tasks require the monkey to remember it over a delay-period, and then make an explicit selection or a “same/different” discrimination based on the previous cue. These tasks typically require memory for one item only. While useful in examining neuronal activity during retention, they are limited in terms of behavioral analysis. They do not examine multi-item working memory effects or examine the impact of the serial position of the match probe on correct report. These factors are important to analyze in that they (1) would provide us with a richer understanding of working memory in monkeys and (2) would allow us to compare monkey behavior more directly to that which has been observed in human subjects.

Monkeys can indeed maintain the memory for multiple items as has been demonstrated in a number of studies. It has been shown that monkeys can remember item sets as large as twenty items over an intervening delay-period (Sands and Wright, 1980). Other studies have demonstrated that monkeys can effectively learn and maintain information for item sequences over delays (Funahashi et al, 1996; Ninokura et al, 2003 and 2004); yet few electrophysiological studies have used multi-item working memory tasks.

More recently, some studies have attempted to begin to shed light on the question of multi-item working memory in monkeys (Inoue and Mikami, 2006; Warden and Miller, 2007 and 2010). In these studies, monkeys were required to remember two items over intervening

delay-periods. Monkeys could adequately maintain information for two items over the delay. However, the tasks employed in these studies were confounded for the additional requirement to remember item sequence and did not vary working memory load.

One study did vary working memory load in monkeys and found that behavioral performance decreased as the number of objects increased (Buschman et al, 2011) consistent to what has been observed in humans. Though this study addresses effects of working memory load, because items were presented simultaneously on the screen the authors could not examine the effects of the serial position of the probe stimulus on behavior.

In the current study we attempted to bridge these gaps by designing a task that would allow us to examine the effects of working memory load and primacy and recency.

To accomplish these goals, we adapted a version of the Sternberg working memory task. Unlike the classic Sternberg task, our task incorporated a cued-recall response (rather than recognition) in order to make the task more difficult for the monkey and produce more errors. Adequate error rates allowed us to examine both accuracy and reaction time as behavioral parameters. Stimulus sets used in this task were basic colors or locations. The advantages of this task are that: (1) it allowed us to vary working memory load (2) it allowed us to examine the effect of the probe's serial position on behavior (3) it was not confounded with the additional requirement to remember sequence (4) allowed us to compare directly the performance of monkeys to humans.

Monkeys exhibited a clear effect of working memory load in that the reaction time was longer and accuracy was lower in the three-item task than under a control condition in which all three samples were the same. They also exhibited a recency effect in that performance was better if the target probe matched the third sample than if it matched one of the first two. The absence of any primacy effects is likely due to the fact that set size was small (three items) and that the

retention interval (time between the final sample and array onset) was short. Previously, it had been demonstrated in both monkey and human subjects that the serial position function is altered such that it is dominated by only a recency effect when the retention interval is less than one second (Wright et al, 1985).

These data demonstrate that monkeys can perform a cued recall version of the Sternberg working memory task. Behavior was strongly modulated by changes in working memory load and the serial position of the probe in a manner consistent with both the human and monkey literature. Having established this, we have shown that this task is an effective and appropriate tool to use in investigating working memory in monkeys. We can now proceed to use this task in interrogating the neural underpinnings of these effects in the monkey brain.

3.0 CORRESPONDENCE OF INFORMATION ACROSS EPOCHS WITHIN A TRIAL

3.1 INTRODUCTION

Working memory is the theoretical construct referring to the set of memory stores that enable us to represent and maintain several items in memory. A vastly accumulating body of research strongly suggests that prefrontal cortex (PFC) is critical in supporting working memory function. Human (for review see Baldo and Shimamura, 2002) and monkey (Fuster, 1989; Goldman-Rakic, 1987; Jacobsen, 1935) lesion studies have demonstrated that damage to prefrontal areas results in working memory impairments. In studies where PFC is intact, it has been reported that PFC activity changes when engaged in working memory tasks. In human PFC, it has been shown that power in the gamma frequency band (30 Hz and higher) of intracranial EEG signals increase when subjects are required to maintain items in memory (Howard et al., 2003). Changes at the population level have also been observed in humans using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). These studies demonstrate that prefrontal regions are selectively activated in tasks utilizing working memory (Fiez et al., 1996; Jonides et al., 1993; Petrides et al., 1993). Much of what we know about

working memory related changes comes from monkey neurophysiological studies. In monkeys, it has been reported that prefrontal neurons are active during delay intervals where information for single items is held in memory (Funahashi et al, 1989; Funahashi et al, 1993; Fuster and Alexander, 1971; Miller et al, 1996). Regardless of the measure, this sustained delay-period activity has been described as the neural correlate of working memory.

The majority of research investigating single unit delay activity involved experiments which required only one item to be remembered, though most complex behaviors require the memory for multiple items. It is important to ask, “how do PFC neurons hold multiple items in working memory”. Three studies have directly addressed this question (Inoue and Mikami, 2006; Warden and Miller, 2007 and 2010). The results of these studies were not entirely consistent with each other and were at times paradoxical possibly due to an unnecessarily complicated task design. The tasks used in these studies not only required the monkeys to remember the two objects but also the sequence in which they appeared. It is known that neurons in PFC are selective for sequences (Barone and Joseph, 1989; Ninokura et al., 2003; Shima and Tanji, 2000). It is not surprising then that previous multi-item working memory studies report that many neurons were selective for both the object identity and the sequence in which it appeared. This may have complicated their interpretations. Also, both tasks used complex objects whose feature space is not easily described. To address these concerns we employed the Sternberg working memory task (1966) which requires the memory of multiple items in the absence of any requirement for sequence memory. Also, this task used simpler stimuli with easily described feature space (location and color) and whose representation in PFC is much better understood. An additional feature that we incorporated into our task design is that

we varied the number of items the monkey had to hold in memory. This feature allowed us to ask questions regarding working memory load and its effect on delay-period activity in PFC.

Drawing from personal experiences in our day-to-day lives, we know that there are limits as to how many items we can remember at one time. Another important question regarding multiple item memory is “why is working memory capacity limited”. Capacity limitation is an important property of working memory. The classic example of this characteristic has been demonstrated by many using the Sternberg working memory task. This task requires subjects to hold in memory a set of numbers to produce a response. It has been observed that as subjects are required to remember more and more items, behavioral performance declines. As described in the previous chapter, we also found this to be true in our experiments. The characteristic of limited capacity implies that PFC has only a certain amount of resources that can be allocated for the memory of items. This suggests that item representations become less robust as a result of depleting resources. Attempts have been made to describe what “resources” means theoretically (Zhang and Luck, 2008; Bays and Husain, 2008), but it has yet to be described what this means in neural terms. One possibility is that information maintained in PFC becomes degraded as items are added to memory. The following experiments determined what was represented during the delay-periods of a multi-item working memory task and if this representation changed as new items were added to memory. If so, how?

3.2 METHODS

3.2.1 Subjects and surgery

Subjects were two adult male rhesus monkeys (*Maccaca mulatta*) weighing approximately 8 and 9 kilograms at the time of the experiments.

Implantation surgery

Surgery was carried out under aseptic conditions. To provide analgesia during surgery preparation (shaving and cleansing the scalp with betadine, endotracheal intubation) monkeys were given atropine (0.4 mg/kg, i.m.) followed by ketamine hydrochloride (20 mg/kg, i.m.) and valium (1.0 mg/kg, i.m.). Monkeys were maintained thereafter on gas anesthesia (isoflurane, 1-2%). The scalp was incised at the midline and the skull surface exposed by retraction of muscles and removal of the periosteum. Titanium bone-screws were implanted around the rim of the exposed skull. A continuous pedestal of rapidly hardening acrylic was built up around the heads of the bone screws so as to cover the exposed skull and embed the head-restraint clamp. The conjunctival membranes were resected and a scleral search coil for monitoring eye position was implanted around the globe of each eye. The leads from each coil were run subcutaneously to a plug on the acrylic pedestal.

Chamber surgery

Chamber surgery was performed once the monkeys were fully trained on the task. Prior to placement of the recording chamber, MRI structural images of the brain were obtained by use of a 4.7-Tesla scanner. The locations of the recording chambers were determined under the guidance of MRI images showing a fiducial marker containing a contrast agent embedded in the cranial implant over lateral prefrontal cortex. A disk of acrylic and skull just large enough to accommodate the recording chamber was removed. The recording chamber, its base flush with the dural surface, was then cemented into the hole with dental acrylic. Chambers were centered approximately 3 mm lateral to the principal sulcus and 5 mm rostral to its posterior tip. The recording chamber for Monkey 1 was located over right PFC. For Monkey 2 the chamber was placed over the left.

3.2.2 Visual stimuli and behavioral task

Behavioral training and monitoring

Monkeys faced a 17 inch LDC monitor in a dark room at a distance of 56 cm while sitting in a primate chair head fixed to a head post. All aspects of the experiment (eye position, monkey's responses, generation and display of visual stimuli, reward delivery) were under on-line control using NIMH Cortex software (provided by Dr. Robert Desimone). Eye position was monitored using scleral search coils (Judge et al., 1980) with a Riverbend field driver and signal processing filter (Riverbend Technologies Inc.). The outputs of the search-coil were led to the computer via an A/D converter and monitored online through NIMH Cortex. The computer sampled and stored horizontal and vertical readings at a rate of 1 kHz.

Daily intake of fluids was regulated in order to maintain motivation to perform the task. Monkeys were trained to perform the cued-recall Sternberg task (for details refer to Chapter 2). Briefly, in one version of the task, the samples were locations and, in the other, they were colors. On each trial three samples were presented in succession for 200 ms. Each sample was followed by a 400 ms delay-period. Then three probes were presented simultaneously. One probe matched a preceding sample. The monkey was rewarded 0.1 cc of water for making a saccade to the match probe. Monkey 1 was trained on both the color and location versions of the task. Monkey 2 was able to adequately learn only the spatial version of the task.

3.2.3 Recording and data collection

Electrode placement

The recording chamber accommodated a plastic plug containing vertical holes spaced 1 mm apart so as to form a square grid (Crist et al., 1988). The grid was inserted and fixed in place with set screws during each recording session. A tungsten electrode with an initial impedance of $\sim 5.0\text{ M}\Omega$ at 1 kHz (Frederick Haer Co.) was advanced through a guide tube using a Narashige hydraulic microdrive affixed to the chamber.

Experimental control of neural data

A second computer ran Plexon software (Plexon Inc.) for on-line waveform analysis and spike recognition. The times of action potentials and other events were sampled at 40 kHz. Data from all trials were displayed on-line and saved to disk. Signals from the electrode were led to the Plexon system, analogue and digital oscilloscopes, and an audio monitor.

Waveform analysis and spike recognition

Raw signals from the amplifier were led to the Plexon waveform analysis program which ran in real time. The system stored templates of action potentials generated by the neuron under study and accepted or rejected each subsequent deflection of the trace on the basis of goodness of match as determined by a template matching algorithm. Further sorting was done off-line using the Plexon Off-line Sorter program.

3.2.4 Data Analysis

Spatial tuning of PFC neurons

We determined the spatial tuning of PFC neurons during the spatial Sternberg task using firing rates from correct control trials where the same sample was repeated three times. We computed the mean firing rate across all three delay-periods for each of the six locations used in the task. These data were then used to create polar plots in MATLAB[®] (Mathworks; Natick, MA) using the `cart2pol` and `polar` functions.

Population analyses

To construct the population histograms we first computed the firing rate in each 1 ms bin, and then averaged firing rates within each bin across neurons. The histograms were smoothed with a 10 ms Gaussian kernel. Only correct trials were used to compute the population histograms and all analyses that follow in this chapter. To compare neuronal activity between preferred and non-preferred conditions we first split the data into odd and even numbered trials. We determined stimulus preference on odd trials. We then categorized even numbered trials using odd-trial-

based selectivity. We avoided circularity in neuron categorization by determining preference on one half of the data and applying these labels to the other. We only included trials for which the preferred or non-preferred sample was also the match probe in a given trial.

We determined whether the average firing rate differed significantly between conditions across the trial, by computing a two-tailed paired t-test over a 10 ms sliding window which was shifted by 1 ms. For each sliding window t-test, each neuron contributed one mean firing rate per condition (preferred/non-preferred). We then took the $-\log$ of the resulting p values and plotted them. We log transformed the p values so that larger values indicated an increase in significance. The role of this step was to provide graphical visualization of the signal timing and not to serve as a test of effect significance.

Statistical tests of single neurons

We carried out a series of ANOVAs to determine which samples (sample 1, sample 2, or sample 3) individual neurons were selective for during each epoch of the trial. We carried out one-factor ANOVAs to determine how many neurons were selective for the color or location of sample 1 during the first delay. During the second delay two-factor ANOVAs determined how many neurons were selective for the color or location of sample 1, sample 2 or both. Multi-way ANOVAs revealed the number of neurons with main effects or interactions of samples 1, 2, or 3 color/location during the third delay. We chose the analysis period for each delay to be 100-600 ms following the onset of the immediately preceding sample.

We also performed tests to see if there was an interaction between the neuron's color/location selectivity and its ordinal position in the trial. On the control trials when the same sample was presented three times, we carried out two-factor ANOVAs where factor 1 was the

sample identity and factor 2 was the ordinal position in which it appeared: first, second, or third sample in the set. Interaction effects from this analysis indicated that the epoch in which the sample was presented had an effect on the neuron's selectivity for that color/location. The two-way ANOVA also determined the number of neurons selective for the epoch of the trial. For all ANOVAs a main-effect or interaction was considered significant if the p value was less than or equal to 0.05.

Correlation analyses

We carried out two separate correlation analyses. The first was to test if neuronal selectivity for a sample persisted across delay-periods. First, we grouped trials based on the first or second sample color/location. Next, we computed mean firing rates during each epoch. We split the data into odd and even numbered trials. This was done so that comparisons could be made between different sets of trials in an effort to eliminate contamination from non-task related effects. Firing rates were normalized using the following procedure: normalized activity ($A(n)'$) in response to a particular color/location (n) is given by:

$$A(n)' = A(n) - \bar{A},$$

where $A(n)$ represents the mean firing rate for a particular color/location and \bar{A} represents the mean firing rate across all n colors/locations. This constrains the firing rates such that the mean across all n equals 0. Each neuron contributes six points to the analysis: the normalized firing rate during the delay for each color/location. We then performed Pearson's correlations between responses to sample 1 during delay 1 (odd trials) to: sample one during delay 2 (even trials) and

sample 1/delay 3 (even trials). Equivalent comparisons were made for sample 2 during delay 2 odd numbered trials. We also compared firing rates on odd and even trials within a single epoch which we expected to be most correlated. This comparison reflected the greatest degree of correlation the neuronal population could produce.

The second correlation analysis tested if selectivity was dependent on the ordinal position of the sample. The ANOVA tested individual neurons for interactions of sample identity and ordinal position. The correlation analysis tests for these effects across the entire neuronal population. We determined the mean firing rate during each delay-period. We then categorized delay-period activity based on the identity of the sample that immediately preceded it. We computed $A(n)'$ for delay-period activity in each category. Using Pearson's correlations we compared the activity across the three delay-periods categorized by the identity of the most recent sample. We compared odd to even numbered trials. Each neuron contributed six data points, its normalized mean firing rate to each color/location. Comparisons of odd and even trial firing rates within a single epoch (reflecting the greatest degree of correlation) were also made.

3.3 RESULTS

We extensively explored both dorso- and ventrolateral PFC while the monkeys performed the Sternberg tasks. We found that only ventrolateral PFC exhibited task related activity. Furthermore, in Monkey 1 from whom we collected data during both the color and spatial tasks, we found that neurons selective for color and location were not segregated but distributed

throughout the ventral portion of the recording region. We also observed that some neurons were selective during both the color and spatial versions of the task. The PFC recording regions of both monkeys were identical though in opposite hemifields. Figure 4 projects the locations of the electrode tracts from which we recorded task related activity onto the MRI structural images from each monkey. We collected 122 neurons during the spatial task and 132 during the color task from Monkey 1; 131 neurons (spatial task only) were collected from Monkey 2.

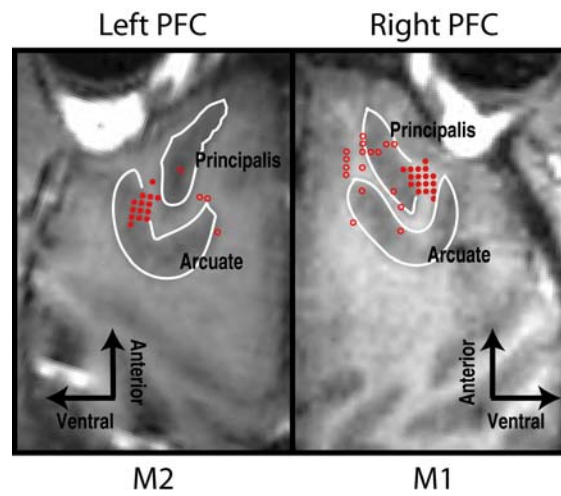


Figure 4. Structural MRI images of cortex underlying the PFC recording chambers

Monkey 1 PFC chamber was located in the right hemisphere; Monkey 2 PFC chamber was located on the left. Filled red circles indicate electrode recording sites where task related neurons were found. Open red circles indicate recording sites where no task-related neurons were identified.

3.3.1 Spatial tuning of PFC neurons

Neurons were tuned to locations contralateral to the recording site. Neurons in the right hemisphere of PFC were tuned for locations on the left side. Neurons in the left hemisphere of

PFC were tuned for locations on the right. The polar plots of delay-period activity during the spatial task for each monkey are shown in Figure 5.

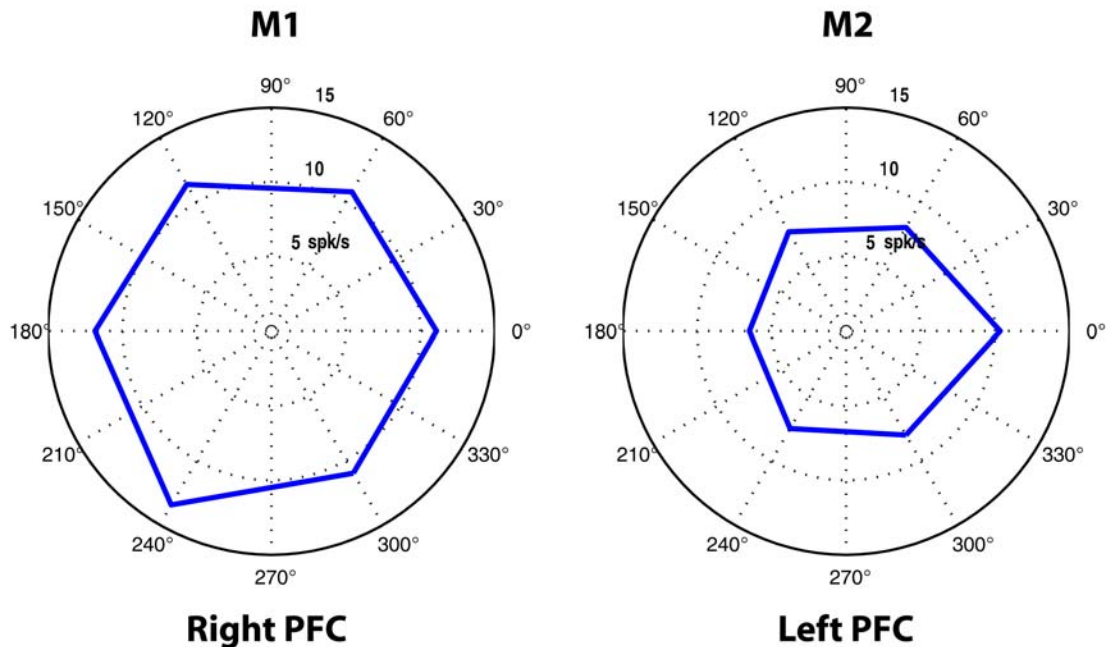


Figure 5. Polar plots of delay-period activity in M1 and M2 during the spatial Sternberg task.

Delay-period activity was averaged across all three delay-periods of the control condition where the same sample location was repeated three times. The dotted concentric circles indicate the average firing rate of the responses at each location.

3.3.2 Population analyses

Using the split halves method described above we plotted the mean activity across the entire trial for the preferred (shown in red) and non-preferred (blue) sample 1, sample 2, and sample 3 stimuli (Figure 6). Only trials on which the match probe corresponded to the preferred or non-preferred stimulus were included. PFC neurons exhibited selectivity for sample identity during the delay. However, the selectivity for a sample was restricted to the delay that immediately

followed. The sample selectivity greatly diminished with subsequent sample presentations in the trial and did not re-emerge at the time of the response.

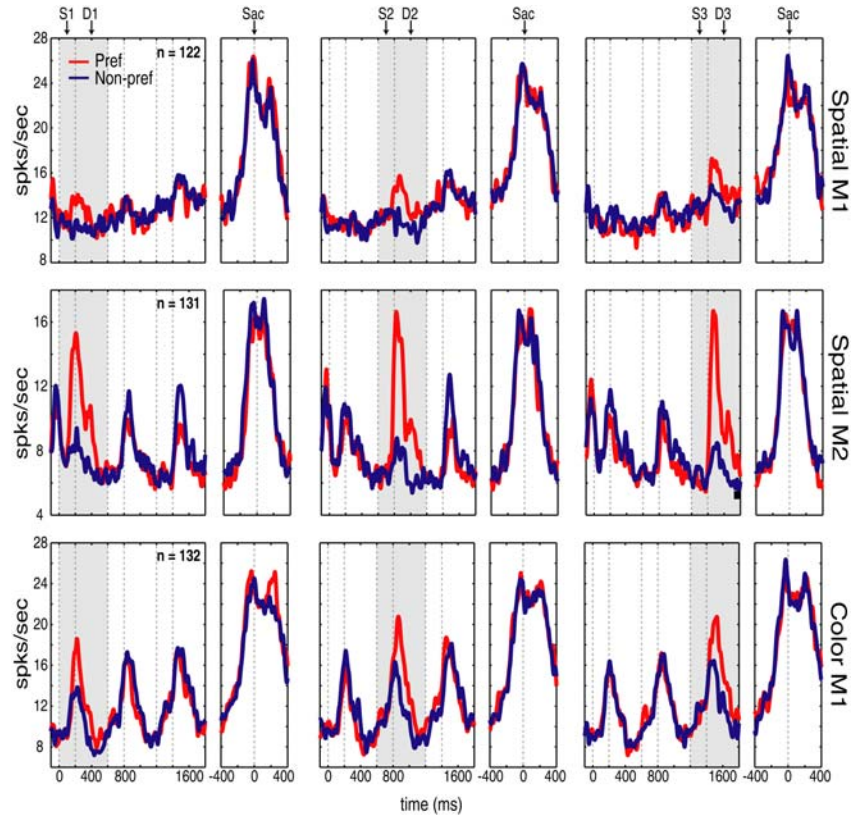


Figure 6. Population histograms of responses to preferred vs. non-preferred samples.

Rows: Population histograms of even numbered trials aligned on the onset of sample 1 and saccade for each monkey. Columns: histograms in the left column are sorted by sample 1 preferred/non-preferred; middle: sample 2 preferred/non-preferred; right: sample 3 preferred/non-preferred. Only trials in which the preferred sample was also the match probe were included. Preference was determined on odd trials and used to categorize even trials plotted above.

Sample selective activity only crossed an arbitrary threshold ($\alpha=0.05$) during the delay that immediately followed. Figure 7 plots the results from the sliding window two-tailed paired t-test. This figure depicts graphically the timing of the preferred/non-preferred population signal across the three epochs of the trial. It does not serve as a test of effect significance. The $-\log$ of

the p values are aligned on the onset of the first sample. The log transformed p values from tests comparing the responses to preferred and non-preferred samples 1 (shown in green), 2 (blue), and 3 (red) across the trial are overlaid on each other. Positive deflections crossing the line of significance are restricted to the delay following the most recently presented preferred or non-preferred sample.

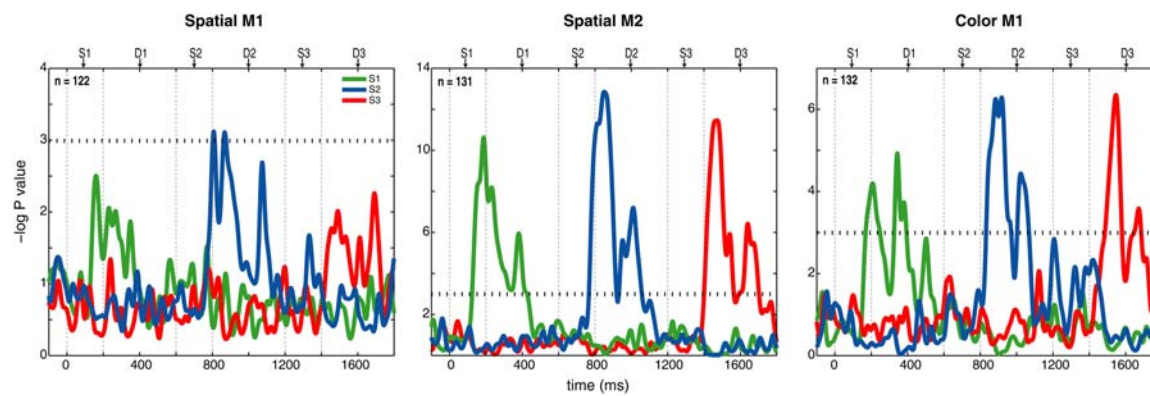


Figure 7. $-\log(p \text{ values})$ from t-test comparing activity when the sample was preferred or non-preferred across the trial

Data are aligned on sample one onset (-100-1800 ms). $-\log(p \text{ values})$ for tests comparing preferred and non-preferred sample 1 are plotted in green, sample 2 in blue, sample 3 in red. The dashed line indicates an arbitrary threshold of $\alpha=0.05$. Threshold crossing is restricted to the delay-period immediately following preferred/non-preferred sample presentation.

3.3.3 Statistical tests of single neurons

Sample selectivity within an epoch

During the delay-period most neurons represented the item that was most recently presented. ANOVA analyses revealed that within a given delay-period, the majority of neurons had a main effect of the immediately preceding sample (Figure 8). Very few neurons exhibited main effects

for samples presented in earlier epochs of the trial. Upon further inspection we found that of these neurons even fewer (6%) continuously exhibited a main effect of sample across more than one epoch of the trial. The population of neurons selective for a previously presented sample within an epoch largely did not overlap with the population representing that sample in earlier epochs. During the first delay, 36% of all neurons had a main effect of sample 1 during the spatial task; 36% during the color task. In the second delay 48% had a main effect of sample 2 during the spatial task; 35% during the color task. In the third delay-period 46% had a main effect of sample 3 during the spatial task; 35% during the color task.

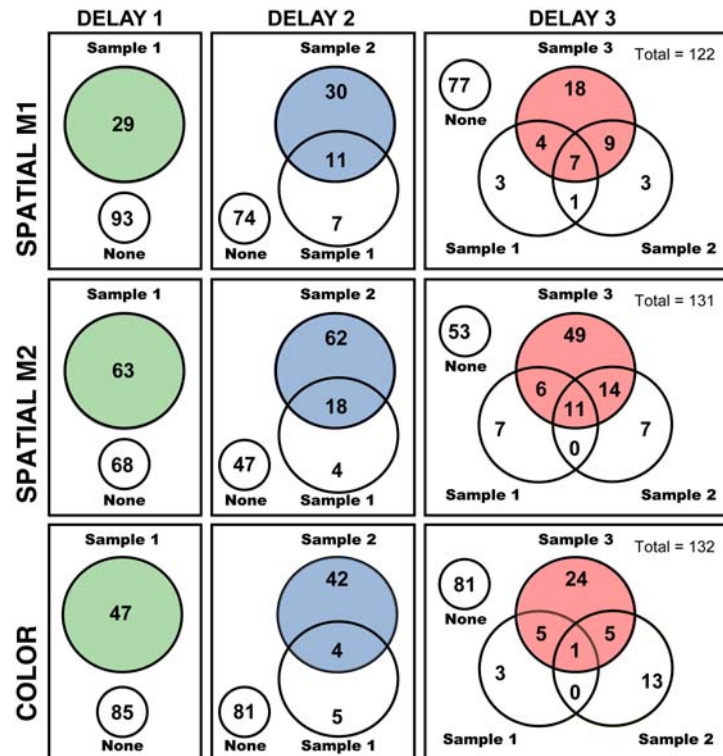


Figure 8. Venn diagrams detailing the number of main effects of samples 1, 2, and 3 within each delay-period for Monkeys 1 and 2 during the spatial and color Sternberg.

Colored circles indicate number of significant ($\alpha=0.05$) main effects contributed by most recent sample. Areas of overlap indicate neurons that showed more than one main effect.

We investigated how the number of neurons representing the interaction of samples 1 and 2 compared to the number of sample 2 main effects. During the second and third delay where the monkey had been presented with two or three items, we found that only a small percentage of neurons represented the combination of two or more samples (Figure 9). During the second delay, 13% of neurons collected during the spatial task and 11% during the color task had a significant interaction effect of samples 1 and 2. During the third delay (not shown in Figure 9), 22% of neurons collected during the spatial task and 26% during the color task had significant interactions of any combination of samples 1, 2, and 3.

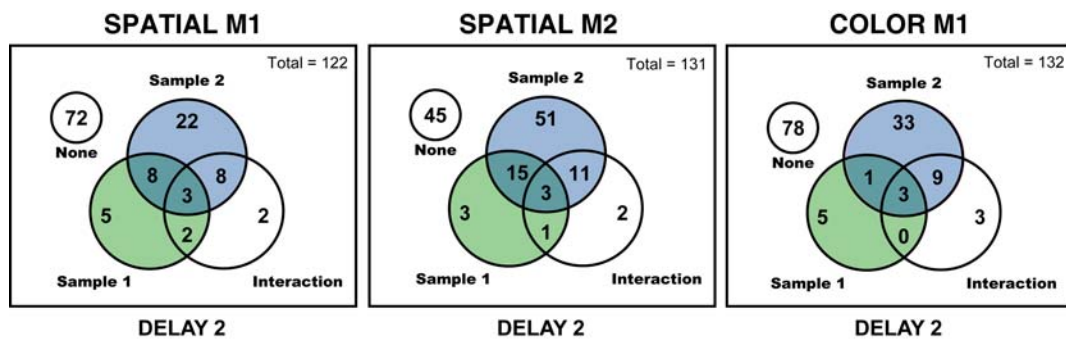


Figure 9. Venn diagrams detailing the number of neurons with main and interaction effects of samples 1 and 2, during the second delay in the spatial and color Sternberg.

Numbers of neurons with main effects of samples 1 or 2 are contained within the green and blue circles, respectively. Numbers of neurons with interaction effects are contained within the white circles. Areas of overlap indicate neurons that showed effects of more than one kind.

We determined how many neurons had a main effect of epoch and if the ordinal position of a sample had an effect on the neuron's selectivity. This was determined on control trials by assessing the number of neurons with main and interaction effects of the sample identity and epoch. The results of the two factor ANOVAs revealed that most main effects were contributed

by the sample identity; there were considerably fewer main effects of epoch. Also, few neurons had a significant interaction effect of epoch and sample identity (Figure 10). We examine this further in the following section using correlation analyses.

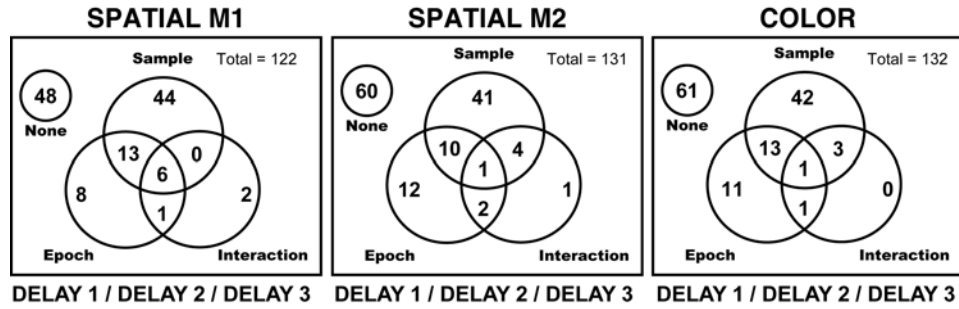


Figure 10. Venn diagrams detailing the number of neurons with main effects and interactions of sample identity and epoch

Venn diagrams describe the results from two factor ANOVAs were computed using the delay-period activity from control trials only. The two factors were epoch (3 levels; first, second, third delay) and sample identity (6 levels: identity of color or location).

3.3.4 Correlation analyses

Sample selectivity across epochs

We tested if the pattern of selectivity for sample one was represented in subsequent delay-periods (delay 2 and delay 3). We also made the equivalent comparison for sample two. We tested this by correlating delay-period activity following the most recent sample with activity in subsequent delays (Figures 11 and 12). We found that the pattern of selectivity for the most recently presented sample was not carried through subsequent delay-periods. This was evidenced by the lack of a positive correlation between sample 1 activity during the first delay and sample 1 activity during the second and third delay-periods. No positive correlations for the equivalent comparisons of sample 2 during the second and third delay-periods were observed. In

fact, correlations between epochs were negatively correlated. This was due to the fact that the delay-period activity was dominated by the most recently presented sample.

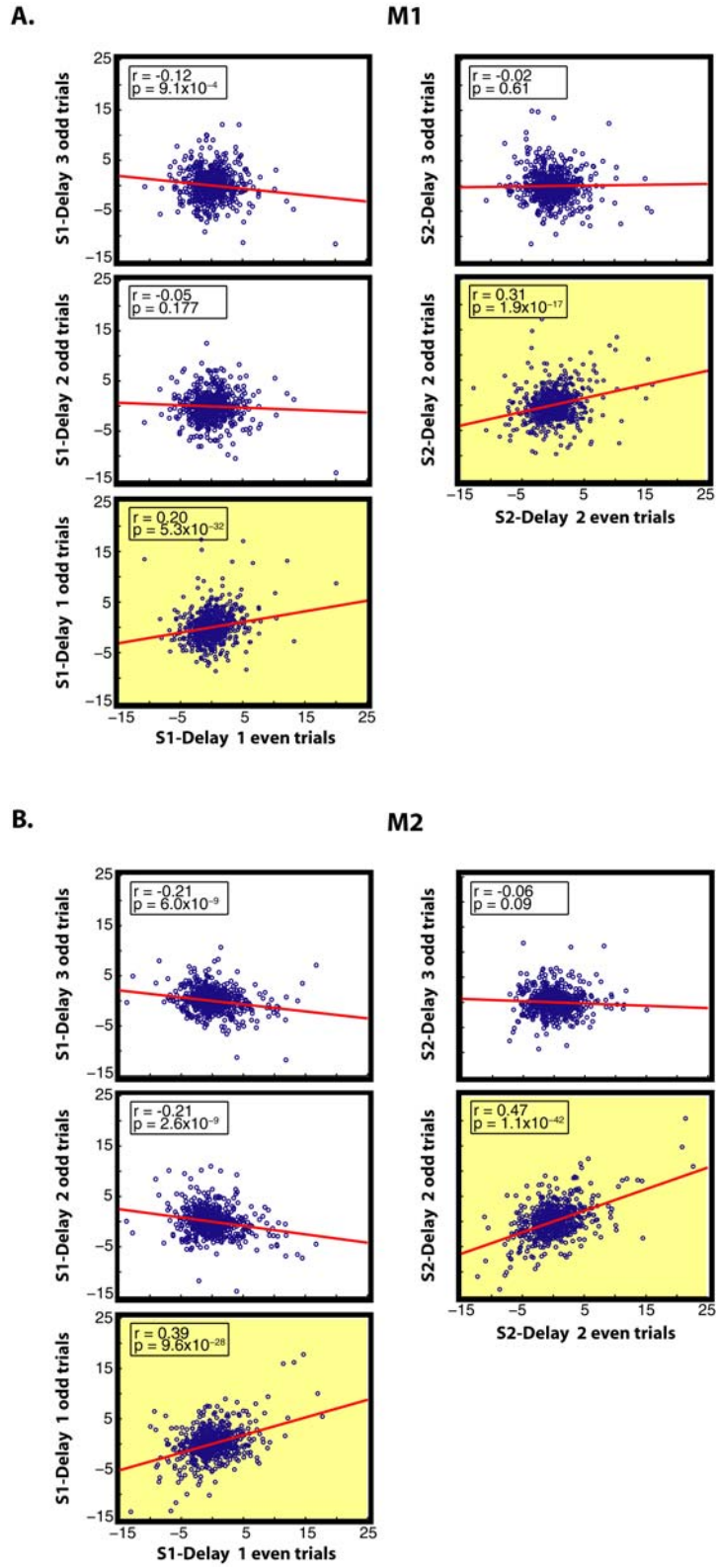


Figure 11. Spatial Sternberg: Correspondence of activity during the current delay with activity in subsequent delays.

Figure 11. (continued)

Scatter plots of the normalized mean responses to samples 1 and 2 within and across epochs (Monkeys 1 and 2). Left column: correlation analyses comparing sample 1 activity during the first delay (even trials) with sample 1 activity in subsequent delays (odd trials). Yellow panels: correlation analysis comparing activity on odd and even trials within an epoch. Right column: equivalent comparisons for sample 2.

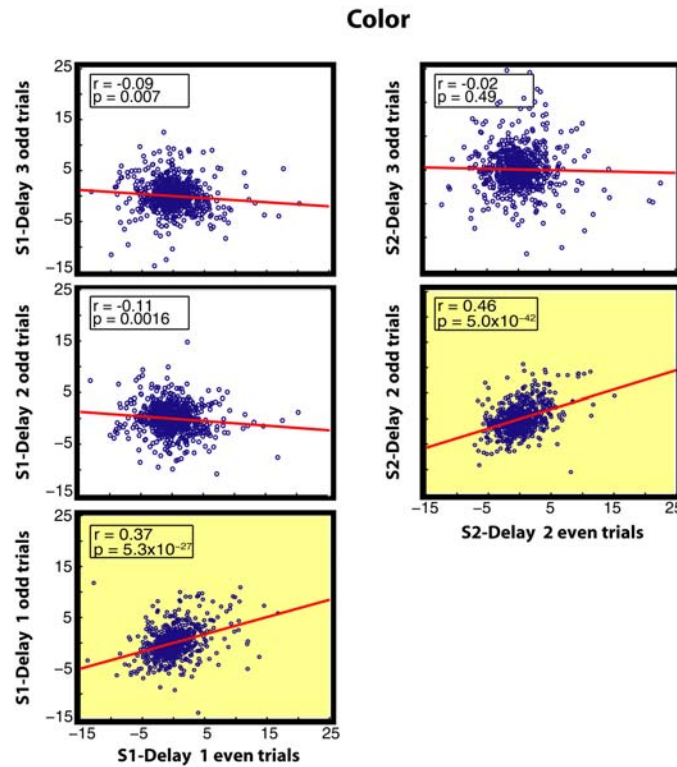


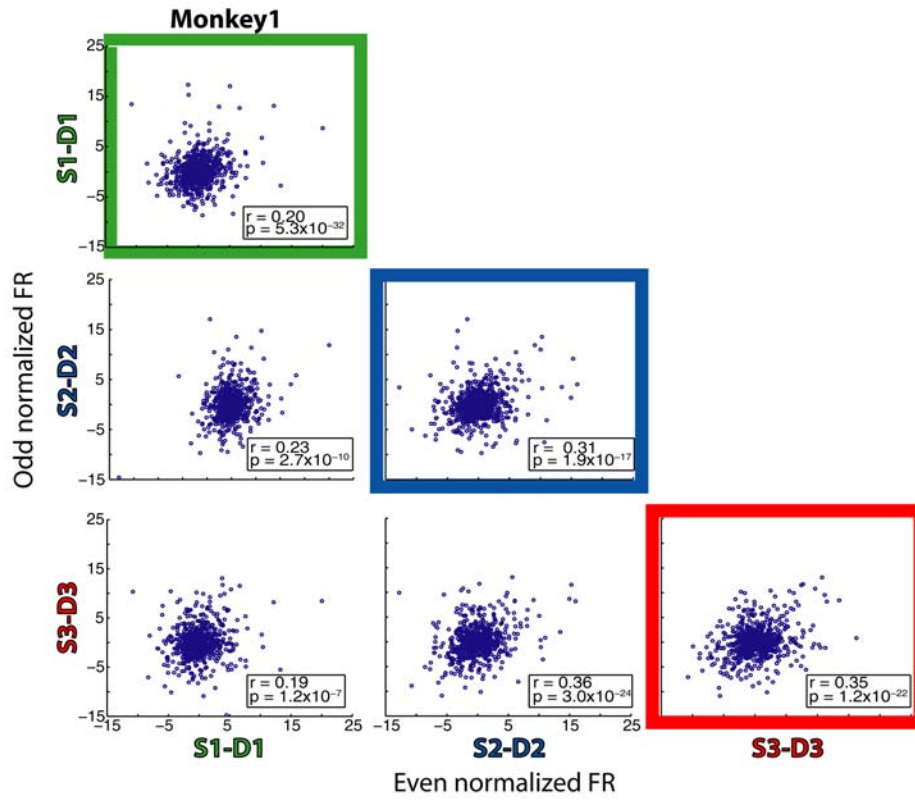
Figure 12. Color Sternberg: Correspondence of activity during the current delay with activity in subsequent delays.

Scatter plots of the normalized mean responses to samples 1 and 2 within and across epochs (Monkey 1 only). Left column: correlation analyses comparing sample 1 activity during the first delay (even trials) with sample 1 activity in subsequent delays (odd trials). Yellow panels: correlation analysis comparing activity on odd and even trials within an epoch. Right column: equivalent comparisons for sample 2.

Sample selectivity between epochs

Correlation analysis revealed that the pattern of selectivity was not affected by the ordinal position of the sample. Figures 13 and 14 describe the results of the correlation analyses comparing patterns of selectivity of the most recently presented samples in the three trial epochs. Pearson's correlation was also carried out to compare odd and even trials within an epoch. The patterns of selectivity on trials within and across epochs were positively correlated and highly significant ($p < 0.0001$). We expected that the selectivity between odd and even trials within an epoch to be the most highly correlated. In general that tended to be the case. We computed the difference in the mean r value within and across epochs. These differences were small and likely to fall within the range of the noise inherent in the signal (mean r value difference between within and across epoch comparisons: M1 spatial=0.02, M2=0, M1 color=0.08). We also tested to see if the selectivity within a delay-period varied between the three trial epochs and found that there were no significant differences ($p < 0.0001$, monte carlo shuffling procedure).

A.



B.

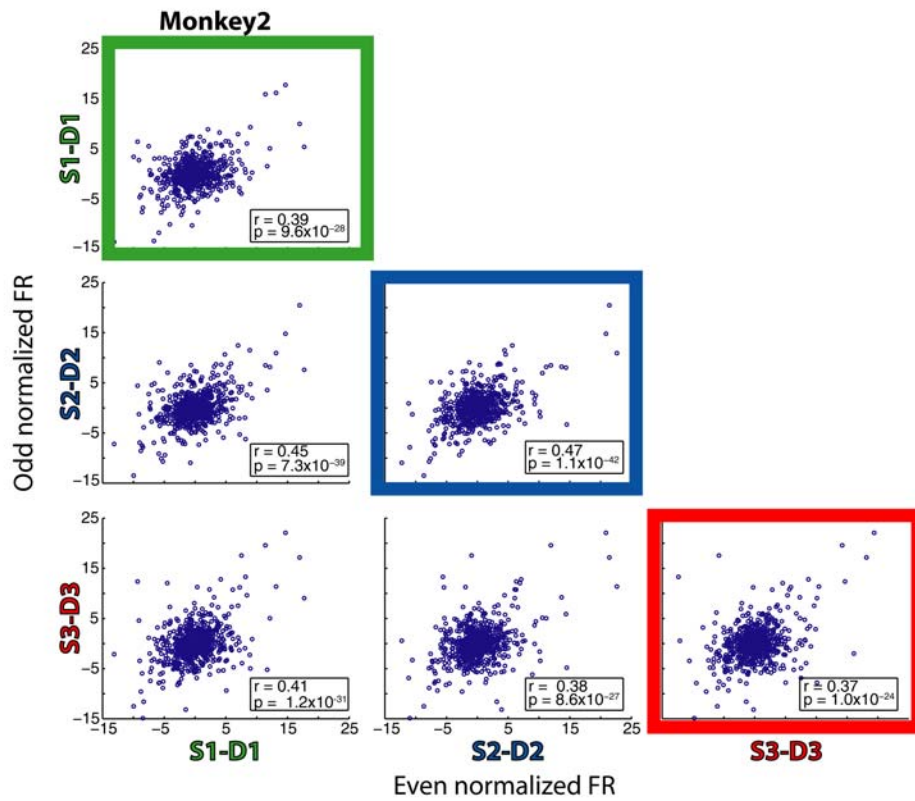


Figure 13. Sample selectivity between epochs of the spatial task.

Figure 13. (continued)

Scatter plots of the normalized mean responses within and across epochs. Each neuron contributed six points corresponding to the normalized mean response to each of the six locations. Comparisons of odd and even numbered trials within an epoch are given in the three colored panels. The remaining three panels show the comparisons between the odd and even numbered trials of different epochs.

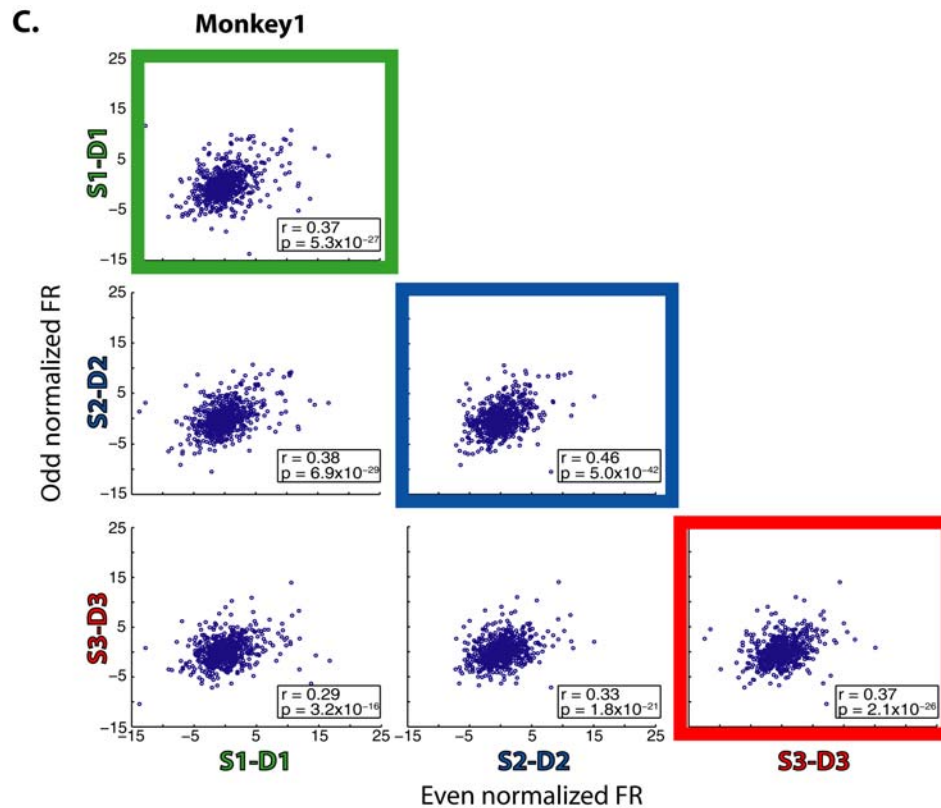


Figure 14. Sample selectivity between epochs of the color task.

Scatter plots of the normalized mean responses within and across epochs. Each neuron contributed six points corresponding to the normalized mean response to each of the six colors. Comparisons of odd and even numbered trials within an epoch are given in the three colored panels. The remaining three panels show the comparisons between the odd and even numbered trials of different epochs.

3.4 DISCUSSION

These results provide insight into how new information affects delay-period activity in PFC in the context of multiple item working memory task. We found that neurons were selective for the color and location of the sample. Delay-period activity most strongly represented the most recently presented sample. In other words, the delay-period activity representing an item is attenuated with subsequent item presentations. There was no correspondence of information carried over the three delay-periods within a trial.

PFC chambers were placed anterior to the arcuate and centered over the principal sulcus. We extensively explored the entire chamber and found that neurons with delay-period activity were restricted to the ventral portion of lateral PFC. This is consistent with what has been reported previously in other working memory experiments (Hasegawa et al., 2004; Inoue and Mikami, 2006; Warden and Miller, 2007 and 2010; Zaksas and Pasternak, 2006). We found neurons selective for both the color and location of the samples within the same region ventral to the principal sulcus. This supports the claim that neurons selective for features of objects and locations are not segregated within lateral PFC (Rao et al., 1997).

During the delay, neurons within the ventral portion of lateral PFC were largely selective for locations contralateral to the recording site. Our data are consistent with previous experiments that examined the spatial tuning of PFC neurons (Funahashi et al., 1989) and found

that the majority of neurons had directionally selective delay-period activity for contralateral locations.

We found that most neurons were not sensitive to the ordinal position of the sample. ANOVA revealed that very few neurons had an interaction effect between the sample identity and the epoch in which it appeared. Furthermore, we found that the ordinal position of the sample did not affect the pattern of selectivity of a neuron. The agreement of selectivity was strong between epochs. This was not surprising because the monkey's only task was to identify which sample appeared in the set of samples recently presented. The monkey was not required to remember the order in which the samples appeared. Because the sequence of items was irrelevant to the monkey we did not expect ordinal position to be strongly represented in delay-period activity.

During a single delay-period neurons were strongly selective for the identity of the sample. We examined if information for sample identity was maintained throughout the course of a trial by (1) examining the time course of sample selectivity across epochs and (2) and determining which sample was most strongly represented during each of the three delay-periods. We found that neurons exhibited strong sample selectivity during the delay. However this selectivity was attenuated with the presentation of subsequent samples in the trial. This was further examined by looking at the main effects of sample identity within each delay-period. We found that the majority of neurons had a main effect of the most recently presented sample during each delay. Very few neurons continuously represented a sample across more than one epoch of the trial. Combined these data show that though the majority of PFC neurons are highly selective for the sample identity during the delay, this signal does not survive subsequent sample presentations. It has been shown previously in monkeys that PFC delay-period activity survives

in the face of subsequent stimulus presentations when these stimuli were displayed as distracters (i.e. the monkey was required to ignore them) and thereby not relevant for the task (Miller et al., 1996). In the context of the current study the monkey was required to remember all of the samples that were presented. Our data show that when the monkey is presented with a sequence of salient stimuli, as was the case in the Sternberg task, delay-period activity representing a recently presented sample is disrupted as new items are added to memory.

Investigation into how neurons represent multiple items in working memory has only recently begun (Inoue and Mikami, 2006; Warden and Miller, 2007 and 2010) and the data from these studies are at times discordant. Previous studies differed in what type of information was most strongly represented by PFC neurons in the delay (sequence, objects, or both). However, all of these studies including our own are consistent in the finding that the selective delay-period activity in PFC is not sustained throughout the trial as one may have expected.

The attenuation of sustained selective responses is not something unique to multi-item tasks. Our data are consistent with the findings from studies investigating PFC in monkeys that employed single-item working memory tasks. These studies have shown that item information severely degraded by the end of the trial and that delay-period activity preceding the monkeys' response could not explain his performance on the task. One study compared activity between visual area MT and PFC during a motion perception delayed match-to-sample task. They found that under conditions when the motion of a random dot pattern was not coherent that unlike MT, PFC delay-period activity period did not correlate with the behavioral response (Zaksas and Pasternak, 2006). Work done by Meyers et al. reported that the accuracy of predicting object identity was strongest during the presentation of the sample and by the middle of the delay the ability to predict the identity of the sample fell below chance. On a single

neuron level, it does not seem that PFC represents item identity long enough for it to be utilized for response selection.

The results we described as well as results from previous studies show that the representation of a sample is not sustained in delay-period activity long enough for it to be utilized for response selection. This presents us with the conundrum that although monkeys were capable of proficient performance on a task requiring the memory for multiple items, delay-period activity in prefrontal neurons cannot easily explain the monkey's working memory behavior. How can these findings be explained?

One possibility is that the memory trace is stored in another part of PFC. The contribution of lateral PFC has been the focus of most working memory research. However, other regions in frontal cortex have been found to demonstrate working memory-related activity. These areas include: the inferior convexity (Romo et al., 1999; Brody et al., 2003), premotor area 6 (e.g., Postle et al., 2000; Awh et al., 1996; Baker et al., 1996; Jonides et al., 1993) and superior frontal areas 6 and 8 (e.g., Postle & D'Esposito, 1999; Mellet et al., 1996; Smith, Jonides, & Koeppe, 1996). Given the extensive literature on working memory, peri-principal prefrontal cortex was a logical candidate to examine multi-item working memory effects. Though the evidence in support of lateral PFC working memory function is strong it cannot be ruled out that working memory may be mediated by other areas in prefrontal cortex.

Another possibility is that delay-period activity in PFC does not strongly represent the memory of sensory items but rather its representations are more complex abstractions of them. It has been shown that one can predict the manipulation of sensory information from the population activity of neurons in PFC. It has been demonstrated that under conditions where the monkey has to mentally manipulate information held in working memory the population activity of

neurons in PFC reflect this mental rotation (Takeda and Funahashi, 2002). It has been shown recently that information for stimulus identities is not very strongly represented in PFC during the delay. This stood in stark contrast to the strength of representations for the task rule which was very strongly represented throughout the delay and into the response period, suggesting that PFC may be a less important structure for representing stimulus properties but highly important for goal and state representations (Meyers et al., 2012). Together these data suggest that PFC may be more important for supporting working memory functions that are extra-mnemonic such as task-set maintenance or the manipulation of sensory information held in working memory

If PFC does not maintain sensory information during working memory tasks, then where is this information stored? Sensory information may be stored in the sensory specific regions of the brain. Several studies have reported working memory related modulations of event related potentials (Ikkai et al, 2010; Vogel and Machizawa, 2004) and BOLD responses (see Wager and Smith, 2003 for a review) that are restricted to posterior regions of the brain. Clinical reports of human lesions to parietal areas describe markedly worse working memory performance when compared to normal controls and patients with lesions to prefrontal cortex (D'Esposito and Postle, 1999). Electrophysiological experiments in monkeys have demonstrated that indeed neurons in a region of parietal cortex (parietal area 7ip) show a delay-period maintenance signal similar to PFC (Chafee and Goldman-Rakic, 1998); though unlike PFC, delay-period activity within this region of parietal cortex is easily disrupted with distracters (Constantinidis and Steinmetz, 1996). Nonetheless, it cannot be ruled that other sensory areas of the brain do not maintain the memory for the features of items.

A final possibility is that the representation of items in working memory is not encoded in the averaged activity of neurons but rather it is encoded within the distributed patterns of

activity across the population of neurons. In the following chapter we will examine the neuronal population activity to further inform our current findings.

4.0 POPULATION CODE FOR MULTI-ITEM REPRESENTATIONS IN PFC DURING THE STERNBERG TASK

4.1 INTRODUCTION

To understand how multiple items are held in working memory we examined single neuron activity in monkey PFC while performing a Sternberg working memory task. We found that PFC neurons did not maintain a sustained representation of an item held in memory throughout the trial. Delay-period activity most strongly represented the most recently presented sample. Adding new items to memory greatly degraded previous representations. The results of the analysis of averaged neural activity leading up to the behavioral response could not adequately explain the monkeys' intact ability to perform the task. Though a great deal of insight can be gained from the investigation of single neuron activity, it is possible that these types of experiments may have overlooked some important aspects of neuronal information processing during the working memory task.

A single neuron only conveys limited amounts of information through a pattern of activity. However, more detailed information can be encoded by aggregating these patterns of activity across many neurons. It is thought that this is the mechanism by which information is

represented in the brain forming the neural code. Extensive theoretical work supports the claim that information in the brain can be represented in the patterns of activity over a distributed network of neurons (Rumelhart et al., 1986; Seung and Sompolinsky, 1993; Zemel et al., 1998).

The assumption most traditional neurophysiological experiments often make is that populations of neurons within an area have the same properties, though in reality the neuronal properties within a population are likely to be more heterogeneous. Population decoding accounts for the unique contributions of each neuron's firing pattern in the service of encoding information. Population decoding methods (Duda et al., 2001; Hung et al., 2005; Quiroga et al., 2006) allow us to pool the activity from many neurons to investigate how and with what accuracy a sensory stimulus or behavioral response can be inferred.

Considering the patterns of activity across groups of many neurons can often lead to deeper insights. It has been demonstrated in PFC and inferotemporal cortex that information carried by the population spanned much longer periods of time than what had been reported in individual neurons (Meyers et al., 2008). The authors also reported that relatively small numbers of neurons encode information in their patterns of activity and this information is passed to other small subsets. Averaging the activity would have obscured or diluted these results.

It has become clear that examining the activity distributed over populations of neurons can provide us with a richer interpretation of information processing in the brain. Using population decoding methods, we asked can sample identity can be effectively decoded across delay-periods in the context of the Sternberg working memory task. Two straightforward ways PFC could encode multiple items are (1) by combining sets of neurons that represent individual sample items (main effect code) or (2) with sets of neurons that are selective for the conjunction of sample items (interaction code) (Figure 15). We determined which code is used by the

neuronal population in PFC using population decoding methods. We also examined if sample representations were sustained by the distributed patterns of activity of the population across the three epochs of the trial.

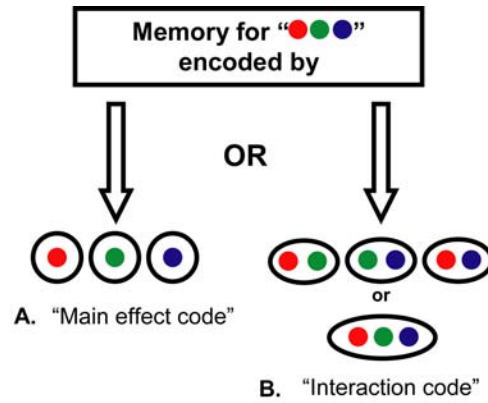


Figure 15. Possible coding schemes for maintaining multiple items.

A. Main effect code: PFC neurons may encode multiple items in working memory by co-activating populations of neurons selective for the individual items within the set. B. Interaction code: multiple items may be encoded by populations of neurons that are selective for the entire set or some subset of items within the set to be remembered.

4.2 METHODS

4.2.1 Subjects and neuronal recordings

Two adult male monkeys (*Maccaca mulatta*) were surgically prepared for neuronal recordings in prefrontal cortex, as previously described (Chapter 3). All procedures and experiments were conducted under the supervision of the University of Pittsburgh Institutional Animal Care and

Use Committee and complied with the guidelines set forth in the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

Data collection methods were identical to those detailed in the previous chapter (Chapter 3). The recording chamber accommodated a plastic plug containing vertical holes spaced 1 mm apart so as to form a square grid (Crist et al., 1988). The grid was inserted and fixed in place with set screws during each recording session. A tungsten electrode with an initial impedance of $\sim 5.0\text{ M}\Omega$ at 1 kHz (Frederick Haer Co.) was advanced through the guide tube using a Narashige hydraulic microdrive affixed to the chamber.

A computer ran Plexon (Plexon Inc.) software for on-line waveform analysis and spike recognition. The times of action potentials and other events were sampled at 40 kHz. Data from all trials were displayed on-line and saved to disk. Signals from the electrode were led to the Plexon system, analogue and digital oscilloscopes, and an audio monitor. The system stored templates of action potentials generated by the neuron under study and accepted or rejected each subsequent deflection of the trace on the basis of goodness of match as determined by a template matching algorithm. Further sorting was done off-line using the Plexon Off-line Sorter tool.

4.2.2 Behavioral Task

Monkeys were trained to perform the cued-recall Sternberg task (for details refer to Chapter 2). Briefly, in one version of the task, the items were locations and, in the other, they were colors. On each trial, three samples were presented in succession, each followed by a delay-period, and then three probes were presented simultaneously. One probe matched a preceding sample. The monkey was rewarded 0.1 cc of water for making a saccade directly to the match probe. Monkey

1 was trained on both the color and location versions of the task. Monkey 2 was able to adequately learn only the spatial version of the task.

Monkeys sat head-fixed in a primate chair facing a 17 inch LCD monitor in a dark room at a distance of 22 inches. All aspects of the experiment (monitoring of single-unit activity, eye position, monkey's responses, generation and display of visual stimuli, reward delivery) were under on-line control using Cortex software provided by Dr. Robert Desimone of the National Institute of Mental Health. Riverbend Instruments systems were used to monitor eye position. The outputs of the search-coil were led to the computer via an A/D converter. The computer sampled and stored horizontal and vertical readings at a rate of 1 kHz. Daily intake of fluids was regulated in order to maintain motivation to perform the task.

4.2.3 Data analysis

We applied a correlation-based population decoding method (Meyers et al., 2010) to evaluate the accuracy of a linear classifier in determining the stimulus that was presented on a given trial. There are several advantages in choosing this method over others. First, because it is correlation based, the computation is fast and has been shown to be empirically equivalent to other perhaps more sophisticated methods such as Poisson naïve Bayes (Meyers et al., 2010). Also, the classifier is invariant to the scalar additions and multiplications of the data. This is a useful feature when comparing classification between different time periods of the trial in which the mean firing rate may have changed, which happens to be the case with the current data set.

The first step was to create a set of pseudo trials to compare with the classifiers. Pseudo trials were made by randomly selecting single trial responses evoked by the same sample.

Pseudo trials were correlated with each classifier and identified as belonging to the class producing the strongest correlation. We examined two straightforward codes PFC could use to support multi-item memory using this method: (1) a main effect code, where separate populations of neurons represent individual sample items or (2) an interaction code, where single populations represent the conjunctions of sample items. Classification accuracy for interaction and main effect decoding was evaluated for each monkey and task. For the remainder of the section the terms “decoding” and “classification” will be used interchangeably. The following sections describe details for each of main effect and interaction classification.

Main effect classifiers and pseudo trials

For main effect decoding we used a set of six classifiers to predict the identities of the most recent sample or previously presented samples within an epoch. For every trial we determined the firing rate during each delay over the epoch 100-600 ms after sample onset. Pseudo trials were constructed by randomly selecting a single trial response from each neuron (Figure 16A). Responses were selected from trials on which the same sample stimulus was shown. We were able to treat the population of neurons as if they were collected simultaneously by creating a population of pseudo response vectors. Ten thousand pseudo trials were used to create a single pseudo population. The mean firing rate of the remaining trials served as the classifier (Figure 16B).

We first tested how accurately the most recent sample could be decoded from delay-period activity. For the rest of the text we will use the words “most recent” to refer to the sample immediately preceding the delay-period. Decoding was performed using classifiers and pseudo populations built from the responses evoked by the most recent sample (Figure 17, top left and

right panels). Next we tested how well samples that were presented in earlier epochs of the trial could be decoded from subsequent delay-period activity. This analysis was performed to determine if information for the sample identity was carried across the trial (Figure 17, top middle panel). We will refer to samples that were presented in earlier epochs of the trial as “previous” for the rest of the text. Before classifying previous samples, it was necessary to remove activity contributed by the most recent sample item. To remove this activity, we preconditioned firing rates by subtracting the mean response to the most recent sample.

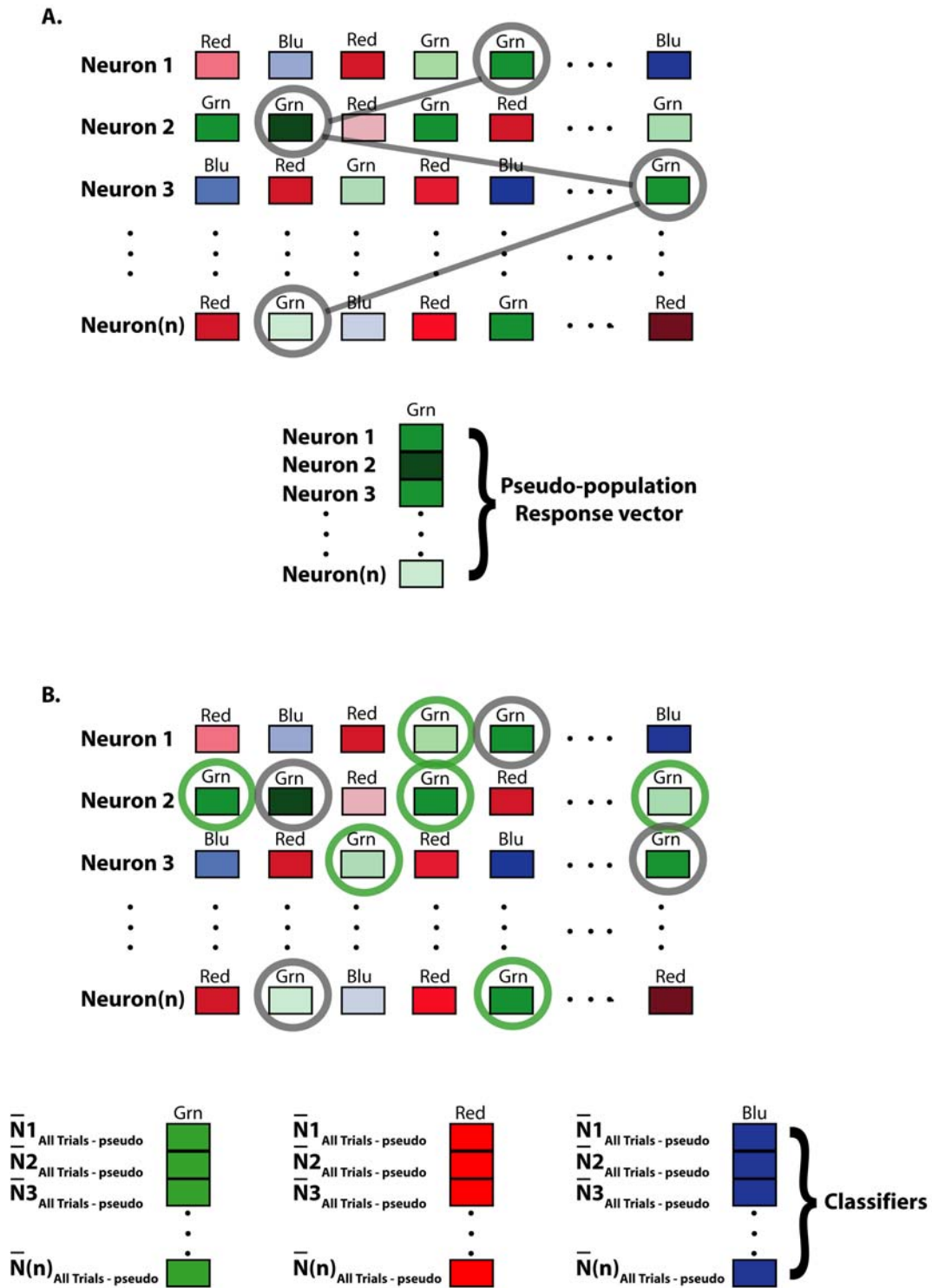


Figure 16. Procedure for building pseudo trials and classifiers

A. Pseudo trial procedure. Each row represents data from neurons collected on separate days. To create a green pseudo trial, a single green trial response is selected randomly from each neuron (circled in grey). B. The mean of the remaining green trials (circled in green) serve as the green classifier. The same procedure is carried out for each stimulus. (Modified from Meyers et al., 2010)

Interaction classifiers and pseudo trials

For interaction decoding we used a set of 30 classifiers to predict the identities of the most recent sample or previously presented samples within an epoch. The set of interaction classifiers corresponded to all possible combinations of the first two samples (Figure 17, bottom). Interaction decoding was restricted to the second delay-period because an equal number of trials for each sample combination was required to avoid classification bias. Counterbalancing was carried out for the first and second samples only. Firing rates were calculated 100-600 ms post stimulus onset for each trial. We created pseudo populations for all sample 1-sample 2 combinations.

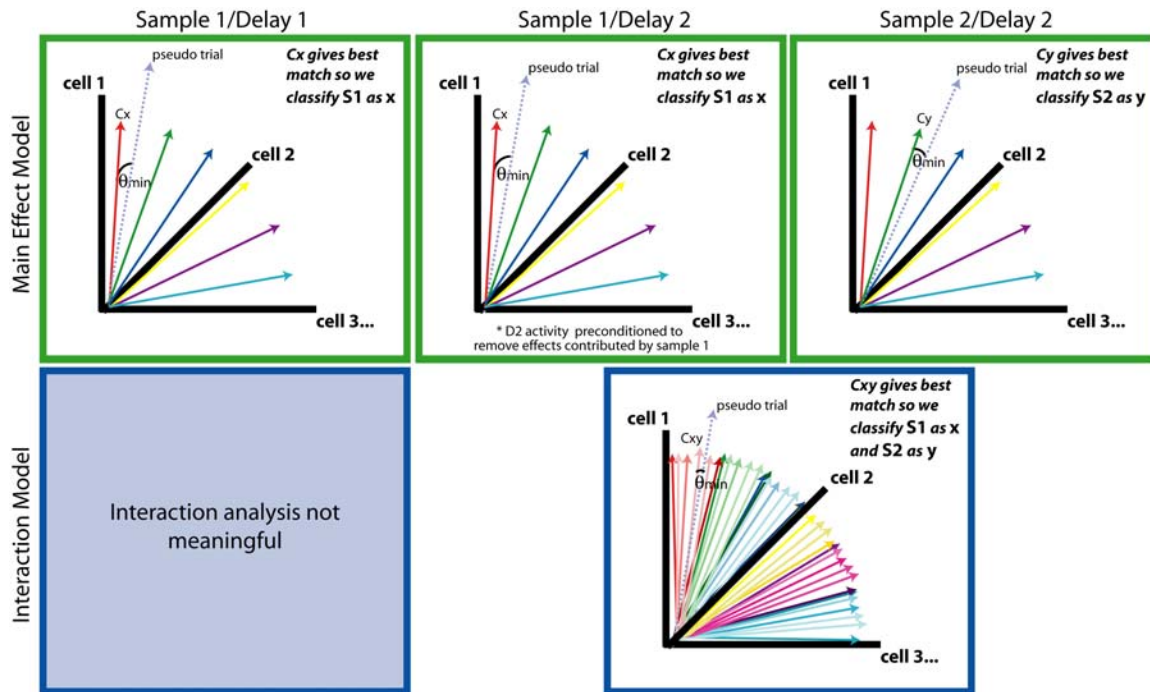


Figure 17. Illustration of main effect and interaction models for decoding.

Classifiers can be considered as vectors in n dimensional space where n corresponds to the number of cells in the pseudo population. The pseudo trial is correlated with each classification vector, C . The correlation coefficient is equivalent to $\cos(\theta)$. The pseudo trial is classified as C_x , C_y , or C_{xy} which correspond to the vectors producing the smallest angle between it and the pseudo trial.

Figure 17. (continued)

Top: Main effect classifiers were used to determine how accurately sample information could be decoded from activity within and across epochs. Left and right panels illustrate the procedure for decoding most recent samples from delay-period activity. The middle panel illustrates decoding procedures for previous sample identities from subsequent delay activity. Bottom: Illustration of interaction model decoding. Classifiers and pseudo trials correspond to combinations of samples 1 and 2. Using the interaction based classifier predictions can be made for sample1, sample2, and the combination of samples 1 and 2.

Decoding procedure

Decoding procedures for the most recent and previous samples were carried out using main effect and interaction-based classifiers. The percentage of pseudo trials most strongly correlated with each classification vector was determined for all pseudo populations. These data were used to construct confusion matrices where the percentage along the diagonal corresponded to correct classifications (Figure 18).

		PSEUDO		
		Red	Grn	Blu
CLASSIFIER	Red	80%	5%	0%
	Grn	10%	85%	5%
	Blu	10%	10%	95%

Figure 18. Decoding performance represented as a confusion matrix.

Consider an example experiment employing three possible stimuli: red, green, blue. The pseudo trial identity is given horizontally and classifiers are labeled vertically. For each of the three classifiers, the percentage of red, green, and blue pseudo trials that it most correlated with is given in each box. The diagonal corresponds to the percentage of times the pseudo trial was identified correctly. If the classifier accurately captures firing rate patterns elicited by a stimulus, the highest percentages would occur along the diagonal of the matrix.

Evaluating significance of classifier performance

The significance of classification accuracy was evaluated using bootstrapping methods. Decoding was repeated 1,000 times producing 1,000 confusion matrices. Classification accuracy on each iteration was defined as the mean percent along the diagonal of each confusion matrix. These means were used to form bootstrap distributions of decoding performance. We would expect the classification accuracy to be 16.67% (1/6) or 3.33% (1/30) if decoding performance was at chance. Decoding performance was considered to be significantly better ($p < 0.0001$) than chance if chance performance fell outside the 95% confidence bounds of the bootstrap distributions.

Classifier performance across increasing population sizes

We evaluated classifier performance across different population sizes to determine the minimum number of neurons needed to accurately predict the sample stimulus from delay activity. We wanted to use the “best case scenario” i.e. where classification was most accurate for this analysis. The main effect decoding of most recent samples was the most optimal. The main effect decoding procedure described above was repeated with neuron populations increased by increments of ten. For each population size we randomly chose a subset of neurons and performed the decoding procedure. This procedure was repeated 1,000 times for each subset to avoid spurious outcomes as a result of randomly choosing a non-representative set of neurons. Bootstrap distributions of decoding performance were created for each subset to evaluate if performance was significantly different than chance.

Comparing decoding accuracy for current and previous stimuli

We compared the performance for predicting most recent samples to the performance of predicting previous samples. We made this comparison for main effect and interaction decoding. The interaction classifier combined information for both the first and second samples. We evaluated separately how well interaction decoding predicted the first or second sample (see Figure 17 bottom panel). We tested if most recent stimuli were more accurately classified than previous stimuli with the following procedure. We iteratively decoded current and previously presented stimuli over the population of N neurons. N neurons were selected randomly with replacement 1,000 times. The percentage of correct classifications was computed with each iteration. This classification procedure was performed on data from each of the three epochs and used to produce six distributions: three distributions of most recent sample classification accuracy and three distributions of previous sample classification accuracy. We determined the accuracy for most recent sample and previous sample predictions to be the mean of the three distributions. We then computed the difference between the classification accuracy for recent and previous samples. This difference was evaluated for significance using a shuffling procedure. The six distributions were combined, shuffled, and then split in half. The mean of each half corresponded to the shuffled recent and previous sample classification accuracy. We computed the difference between the shuffled means and repeated this procedure 1,000 times producing a distribution of shuffled differences. The real performance difference was compared to the shuffled distribution. Performance differences were considered to be significant ($p < 0.0001$) if it was outside the 95% confidence bounds of the shuffled distribution.

Comparing main effect and interaction decoding models

We compared the performance of main effect decoding to interaction-based decoding for identifying sample one or sample two from the second delay using a procedure similar to the one described above. Two bootstrap procedures were carried out for determining the accuracy for predicting sample one during the second delay: one using main effect and one using interaction decoding. We also carried out two decoding bootstrap procedures to determine the accuracy for predicting sample two from the second delay using the two classifiers. We computed the difference between main effect and interaction decoding accuracy. We determined if main effect decoding was better at identifying samples one or two from the second delay by using a shuffling procedure similar to the one described above. Independent shuffling procedures were carried out for decoding samples one and two. The interaction and main effect distributions were shuffled, and then split in half. The mean of each half corresponded to the shuffled accuracy of main effect and interaction-based decoding. We computed the difference between the two shuffled means and repeated this procedure 1,000 times to produce a distribution of shuffled differences. The real accuracy difference was compared to the shuffled distribution. Performance differences between the two types of decoding were considered to be significant ($p < 0.0001$) if it was outside the 95% confidence bounds of the shuffled distribution.

4.3 RESULTS

4.3.1 Main effect classifier

Figure 19 A. shows the accuracy levels obtained for identifying the most recent (shown in the colored boxes) and previous samples (black boxes) with the main effect classifier. The ability of the classifier to decode the most recent sample was significantly above chance ($p < 0.0001$) (average percent correct across the three samples for Monkey 1: 35%, Monkey 2: 58%, Monkey 1 color: 53%). Identifying previous samples from activity in subsequent delays was far less accurate (average percent correct for Monkey 1: 25%, Monkey 2: 27%, Monkey 1 color: 23%). Over the second and third delay-periods performance of the main effect classifier dropped by as much as half when classifying previously presented samples. This drop in performance accuracy observed for decoding samples that were presented earlier in the trial was significant ($p < 0.0001$). The results of the shuffle-based significance test for comparing most recent to previous sample classification performance are given in Table 1.

Classifier performance across increasing population sizes

We wanted to determine the smallest population that could accurately decode the most recent sample. We found that the minimum number of neurons required to accurately predict the identity of the most recent sample varied between the two monkeys and tasks. When classifying data collected from Monkey 1 during the spatial task, a minimum of 100 neurons was required to

accurately identify the most recent sample (Figure 19 B. left panel). Far fewer neurons (twenty) from Monkey 2 were needed to accurately decode sample identity (Figure 19B middle panel). When using data collected from Monkey 1 during the color task, sixty neurons yielded accurate classification (Figure 19 B. right panel). The variability in the number of neurons required to effectively decode sample identity across the monkeys and tasks is likely due to differences in the depth of selectivity exhibited by these neurons. Neurons collected from Monkey 1 were less selective than those collected from either Monkey 2 during the spatial task or Monkey 1 during the color task.

4.3.2 Interaction classifier

Performance of the interaction classifier was poor at predicting combinations of samples during the second delay (classifier performance for M1 spatial task: 6.4%, M2 spatial task: 11.4%, M1 color task 7.0%). Though classification accuracy was low for Monkey 2 the performance of the classifier was statistically better ($p < 0.0001$, bootstrap) than chance (3.33%). The performance of the interaction classifier was significantly ($p < 0.0001$) worse than main-effect decoding in identifying either sample one or sample two during the second delay for both monkeys and both tasks. The results of the shuffle-based significance test comparing main effect versus interaction-based sample identification performance is given in Table 2. The ability of the interaction classifier was worse when compared to the main effect classifier. However, the relative ability to better identify the current sample stood true for interaction-based decoding as well (Table 1 and Figure 19 C.).

Table 1. Comparison of current versus previous sample classification accuracy

	Classifier Acc _{most recent} - Classifier Acc _{previous}		
	M1 Spatial	M2 Spatial	M1 Color
Main Effect Classifier	9.3 % *	31.3 % *	30.0 % *
Interaction Classifier	7.1 % *	16.0 % *	13.9 % *

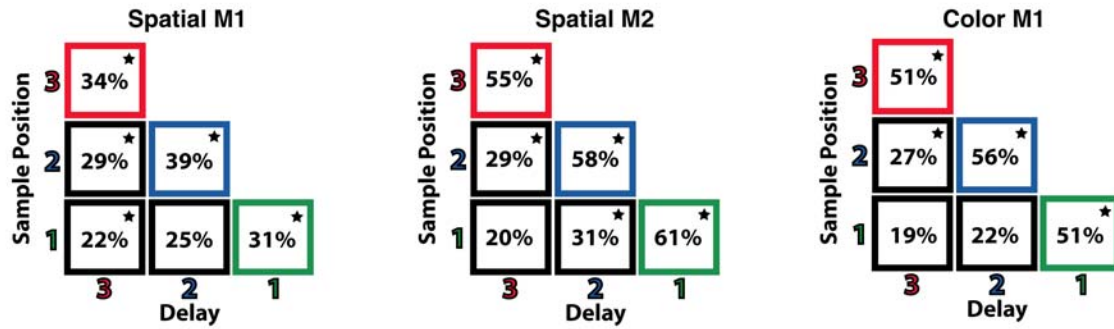
* denotes $p < 0.0001$

Table 2. Comparison of main effect versus interaction based classification

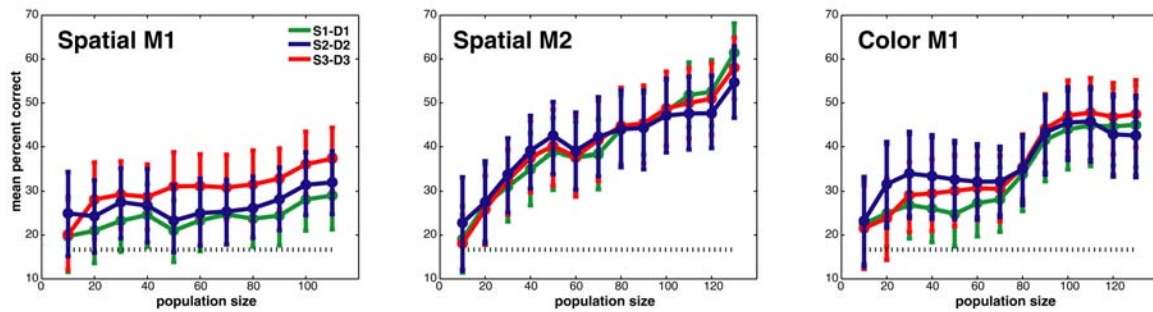
	Classifier Acc _{ME} - Classifier Acc _{Interxn}		
	M1 Spatial	M2 Spatial	M1 Color
S1 identity from D2	5.3 % *	7.3 % *	1.6 % *
S2 identity from D2	11.6 % *	18.2 % *	22.4 % *

* denotes $p < 0.0001$

A. Main Effect Model



B. Classifier Accuracy Across Population Sizes



C. Interaction Model

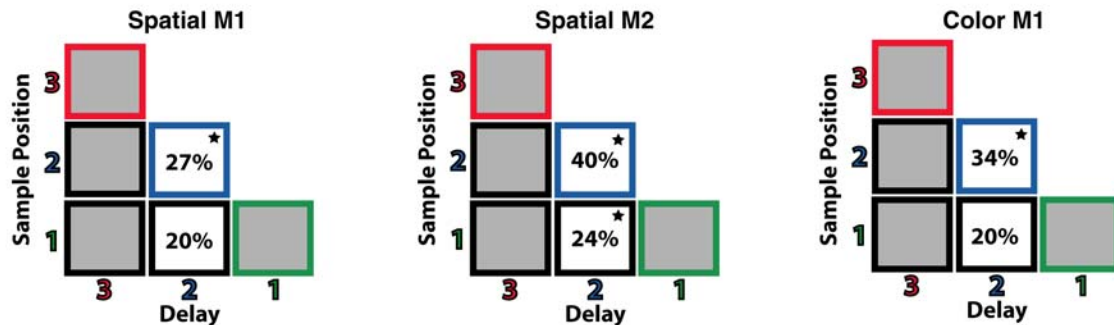


Figure 19. Summary of decoding analyses

A. The results of main effect classification for Monkeys 1 and 2 in the spatial task and Monkey 1 color task. The accuracy of identifying the current sample are given in the colored boxes (green: sample1/delay1, blue: sample2/delay2, red: sample3/delay3). Accuracy of reporting previous samples are given in the black boxes. B. Classifier accuracy over increasing population sizes. Analysis was performed for classification of the current sample only. Error bars represent 95% confidence intervals. The dashed line indicates chance performance (16.67%). C. Results of the interaction based classifier. There were 30 interaction classifiers corresponding to all possible sample 1/sample 2 combinations. Because only samples one and two were counter balanced current and previous sample decoding could only be evaluated for the second delay. * indicates significantly different from chance ($p < 0.0001$ bootstrap test)

4.4 DISCUSSION

In the previous chapter we examined how stimuli were represented in the average activity of prefrontal neurons during a multi-item working memory task. Analyses based on averaged single unit activity revealed that the identity of the most recently presented sample was represented most strongly during the delay and that information for previous samples became greatly degraded as more items were added to memory. The results based on the averaged activity over the population of PFC neurons could not explain the monkeys' performance on the task. It is a widely held belief that information in the brain is represented in the patterns of activity over a distributed network of neurons. We considered the possibility that representations of items held in memory were not represented in the averaged population activity but in the activity across the population of neurons.

Results from the population decoding analyses were largely concordant with what we had observed previously using traditional analysis methods: within a given epoch, the representation of the most recently presented sample was strongest. The accuracy of decoding the identity of the most recent sample was significantly better than chance even when classifying a population of neurons as small as twenty. Furthermore, delay-period activity strongly represented the identity of single samples and not the combination of samples presented in a trial. We also used interaction-based classification to categorize single sample identities. The performance of interaction-based decoding was significantly worse at predicting single samples when compared to main-effect, though like the main effect classifier it was relatively better at predicting the most

recent sample. Combined, these data show that population activity: (1) strongly represented the identity of individual samples and not sample combinations and (2) most strongly represented the most recent sample.

The representation of the identity of early samples diminished significantly as the trial progressed though there was some evidence that information for samples presented in previous epochs of the trial was represented later in the trial. Despite this significant loss of information for sample identity as new items are added to memory, we cannot completely rule out that these weak representations are sufficient to support working memory. We can say however, that classifier performance in predicting earlier samples from delay-period activity was significantly worse when compared to performance in predicting the identity of the most recently presented sample. Given that these representations are weak and cannot be identified reliably across epochs of the trial, it would be highly unlikely that these weak representations are enough to support multi-item working memory. If this is indeed the case, we are faced with the conundrum that although monkeys were capable of proficient performance on a multi-item working memory task, the delay-period activity distributed across the population of prefrontal neurons could easily explain the monkey's working memory behavior. How can these findings be explained?

It is possible that representations of sensory information are not stored in PFC. Other studies have reported that stimulus information represented in the activity of neurons is significantly degraded by the end of the delay-period. Meyers et al. reported that the accuracy of predicting object identity was strongest during the presentation of the sample and by the middle of the delay the ability to predict the identity of the sample fell below chance. It has also been observed that during a motion perception match-to-sample task, PFC delay-period activity was transient and did not correlate with the behavioral response (Zaksas and Pasternak, 2006). These

data combined with our own show that PFC does not represent item identity long enough for it to be utilized for response selection and may therefore not be a primary structure for supporting the storage of feature information during working memory tasks. Evidence that suggests that PFC may be more important for supporting working memory functions that are extra-mnemonic such as task-set maintenance (Meyer et al., 2012) or the manipulation of sensory information held in working memory (Takeda and Funahashi, 2002).

Storage may be supported by other areas of PFC. Delay-period activity is not unique to lateral prefrontal cortex. The inferior convexity (Romo et al., 1999; Brody et al., 2003), premotor area 6 (e.g., Postle et al., 2000; Awh et al., 1996; Baker et al., 1996; Jonides et al., 1993) and superior frontal areas 6 and 8 (e.g., Postle & D'Esposito, 1999; Mellet et al., 1996; Smith, Jonides, & Koeppe, 1996) have all been found to demonstrate working memory-related activity. Posterior regions of the brain have also been shown to have delay-period activity. Studies have observed working memory related changes in event related potentials (Ikkai et al., 2010; Vogel and Machizawa, 2004) and BOLD responses (see Wager and Smith, 2003 for a review) that occur over posterior regions and often times in the absence of any changes in PFC. Clinical reports of humans who have sustained lesions to parietal areas have described markedly worse working memory performance when compared to normal controls and patients with lesions to prefrontal cortex (D'Esposito and Postle, 1999). Electrophysiological experiments in monkeys have also reported that neurons in a region of parietal cortex (parietal area 7ip) show a delay-period maintenance signal similar to PFC (Chafee and Goldman-Rakic, 1998). These data demonstrate that other areas do show working memory related activity and that damage to these areas can result in working memory performance deficits. Though the evidence in support of

lateral PFC working memory function is strong it cannot be ruled out that working memory may be mediated by other areas of the brain.

A final possibility we consider is that by not collecting the data simultaneously that information inherent in the correlated activity between neurons within the population was lost. Pseudo trials were used to simulate simultaneously collected data from data that were collected in separate sessions. It is known from data that were collected simultaneously that the noise within the neural signal is often correlated across the population of neurons on a trial by trial basis. Some suggest these correlations can carry information (Averbeck et al., 2006; Averbeck and Lee, 2006). This method assumes that noise correlations between neurons are not important for conveying information though that may not be the case. Though pseudo populations keep the stimulus induced aspect of the neural population code intact, they destroy noise correlations between neurons that occur on a given trial. Studies have compared the accuracy of decoding data that were collected simultaneously to data that were collected in separate sessions and report that there is no difference (Aggelopoulos et al., 2005; Anderson et al., 2007; Panzeri et al., 2003). However, until more is known about the importance of noise correlations for information processing, we cannot rule out that making the assumption that it is not may have distorted our results.

5.0 STRENGTH OF SELECTIVITY DURING THE DELAY AND PERFORMANCE

5.1 INTRODUCTION

Delay-period activity is the most important neural correlate of working memory because: (1) this activity persists in the absence of an overt stimulus, (2) the persistent activity is selective for items held in memory, and (3) it is resistant to distracters. Electrophysiological experiments in monkeys revealed that firing rates of PFC neurons increase during the delay of delayed response tasks (Funahashi et al, 1989; Fuster and Alexander, 1971; Kubota and Niki, 1971; Miller et al, 1996). Human functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies have reported similar sustained increases in PFC at the population level while subjects performed a working memory tasks (Fiez et al., 1996; Jonides et al., 1993; Petrides et al., 1993). The data from these experiments and many more that followed have lead to the conclusion that prefrontal cortex holds information on the basis of which decisions are made.

It has been shown that PFC delay-period activity is selective for many different types of features such as visual (Funahashi et al., 1989; Inoue and Mikami, 2006; Warden and Miller, 2007), auditory (Bodner et al., 1996) and somatosensory (Romo et al., 1999). PFC delay-period

activity is not only selective for individual stimulus features (e.g. shape and location) (Wilson et al., 1993) but has been shown to exhibit more complex selectivity such as combinations of certain features (Rao et al., 1997) as well as more abstract information such as task rules (e.g. match vs. non-match) (Meyers et al., 2012; Wallis et al., 2001; White and Wise, 1999) and sequence (Funahashi et al., 1997; Ninokura et al., 2003 and 2004).

If information is to be represented in the service of working memory it is important for these representations to be able to survive distractions. Miller et al. (1996) demonstrated that signals carried during the delay-period in PFC are maintained even when monkeys were presented with distracters. This was in contrast to what they observed in inferotemporal cortex (IT), where distracters were shown to attenuate stimulus selective activity during the delay.

For these reasons it is widely supported that the information carried in delay-period activity is important for response selection during a working memory task. If this is the case, a logical assumption is that errors in a working memory task are the result of weakened item representations during the delay. Previous studies have shown that delay-period activity on error trials is either absent or truncated (Funahashi et al., 1989; Fuster, 1973, Inoue and Mikami, 2006; Sawaguchi and Yamane, 1999). It has also been shown that disrupting PFC delay-period activity via electrical stimulation during the delayed match-to-sample task reduced neuronal selectivity and produced behavioral deficits (Sobotka et al., 2005). Authors of this experiment concluded that the memory trace is carried in PFC activity and that disruptions of activity during the delay corrupted the memory trace resulting in the observed performance deficits. If this claim were true it should be possible to predict the accuracy of a response based on delay-period activity. Pessoa et al. (2002) demonstrated that indeed trial-by-trial performance could be predicted from delay-period BOLD responses in humans performing a working memory task. Our goal was to

determine if performance was reflected in delay-period activity by demonstrating that selectivity during the delay-period was weaker when performance was poor. Using the Sternberg working memory tasks we determined whether there was a correlation between the strength of the neuronal representation of a stimulus during the delay and two measures of performance: accuracy and reaction time.

5.2 METHODS

5.2.1 Subjects and recordings

Two adult male rhesus monkeys (*Maccaca mulatta*) (8 and 9 kg) were surgically prepared for neuronal recordings in prefrontal cortex, as previously described (Chapter 3). All procedures and experiments were conducted under the supervision of the University of Pittsburgh Institutional Animal Care and Use Committee and complied with the guidelines set forth in the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

Data collection methods were identical to those detailed in the previous chapter. The recording chamber accommodated a plastic plug containing vertical holes spaced 1 mm apart so as to form a square grid (Crist et al., 1988). The grid was inserted and fixed in place with set screws during each recording session. A tungsten electrode with an initial impedance of ~ 5.0 M Ω at 1 kHz (Frederick Haer Co.) was advanced through the guide tube using a Narashige hydraulic microdrive affixed to the chamber.

A computer ran software for on-line waveform analysis and spike recognition (Plexon). The times of action potentials and other events were recorded with 1 msec resolution. Data from all trials were displayed on-line and saved to disk. Signals from the electrode were led to the Plexon system, analogue and digital oscilloscopes, and an audio monitor. The system stored templates of action potentials generated by the neuron under study and accepted or rejected each subsequent deflection of the trace on the basis of goodness of match as determined by a template matching algorithm. Further sorting was done off-line using the Plexon Off-line Sorter tool.

5.2.2 Behavioral task

The two monkeys were trained to perform the cued-recall Sternberg task (for details refer to Chapter 2). Briefly, in one version of the task, the items were locations and, in the other, they were colors. On each trial, three samples were presented in succession for 200 ms, each followed by a 400 ms delay-period. Then three probes were presented simultaneously. One probe matched a preceding sample. The monkey was rewarded 0.1 cc of water for making a saccade directly to the match probe. Monkey 1 was trained on both the color and location versions of the task. Monkey 2 was able to adequately learn only the spatial version of the task.

The monkeys faced a 17 inch LDC monitor in a dark room at a distance of 58 cm. Eye position was monitored using scleral search coils (Judge et al., 1980) with a Riverbend field driver and signal processing filter (Riverbend Technologies Inc.). The outputs of the search-coil were led to the computer via an A/D converter and monitored online through NIMH Cortex (provided by Dr. Robert Desimone). The computer sampled and stored horizontal and vertical readings at a rate of 1 kHz. All aspects of the experiment (eye position, monkey's responses,

generation and display of visual stimuli, reward delivery) were under on-line control using NIMH Cortex. Daily intake of fluids was regulated in order to maintain motivation to perform the task.

5.2.3 Data Analysis

Population histograms were constructed by computing the firing rate in each 1 ms bin of the trial. The data were averaged and the resulting histograms were smoothed with a Gaussian kernel ($\sigma = 10$ ms).

5.2.3.1 Correct versus error trials

Population histograms were computed for correct and incorrect trials. We determined the most and least preferred stimuli for each epoch of the trial. Selectivity was based on correct trials only. Preferred and non-preferred error trials were sorted using correct-trial-based selectivity. We only included trials for which the preferred or non-preferred sample was also the match probe on a given trial.

Discriminative signal

The discriminative signal reflects the depth of selectivity in the neuron's firing pattern. It was defined as the difference in responses to preferred and non-preferred samples in each epoch. These correspond to the black traces in the bottom panels of Figures 20 and 21. We computed the mean discriminative signal over 100-600 ms following the onset of samples 1, 2 and 3. For each sample we compared correct and error mean discriminative signals in the three trial epochs

using a two-tailed paired t-test. For each t-test, each neuron contributed one mean discriminative signal per condition (correct/error).

5.2.3.2 Fast versus slow reaction time trials

Error trials were excluded from this analysis. We ordered trials based on reaction time (RT) from shortest to longest. We labeled the top third as “fast” and the bottom third as “slow” RT trials. We determined the most and least preferred sample 1, 2 and 3 and computed histograms for each condition. Again, we only included trials for which the preferred or non-preferred sample was also the match probe.

Discriminative signal

We computed the fast and slow discriminative signals for samples 1, 2, and 3 (black traces in the bottom panels of Figures 22 and 23). The mean discriminative signals 100-600 ms following the onset of samples 1, 2 and 3 were computed. For each sample we compared fast and slow mean discriminative signals in the three trial epochs using a two-tailed paired t-test. For each t-test, each neuron contributed one mean discriminative signal per condition (fast/slow).

5.3 RESULTS

We collected a total of 253 neurons during the spatial Sternberg task and 132 neurons during the color task.

5.3.1 Selectivity on correct vs. error trials

Selectivity for sample identity was evident on both correct and error trials and did not persist into subsequent delays. Correct trial discriminative signals were more sustained within an epoch. The depth of selectivity was also greater on correct trials. Two-tailed paired t-tests revealed significant differences ($p < 0.05$) between correct and error discriminative signals. Significant differences were largely restricted to the current epoch (Figures 20 and 21). Monkey 1 performed very well on the spatial Sternberg task and had few error trials to contribute to this analysis (<17%). In particular, Monkey 1 very rarely made errors when the match probe matched the third sample. This accounts for the difference in signal-to-noise apparent between correct and error trial histograms particularly evident for later samples in Monkey 1.

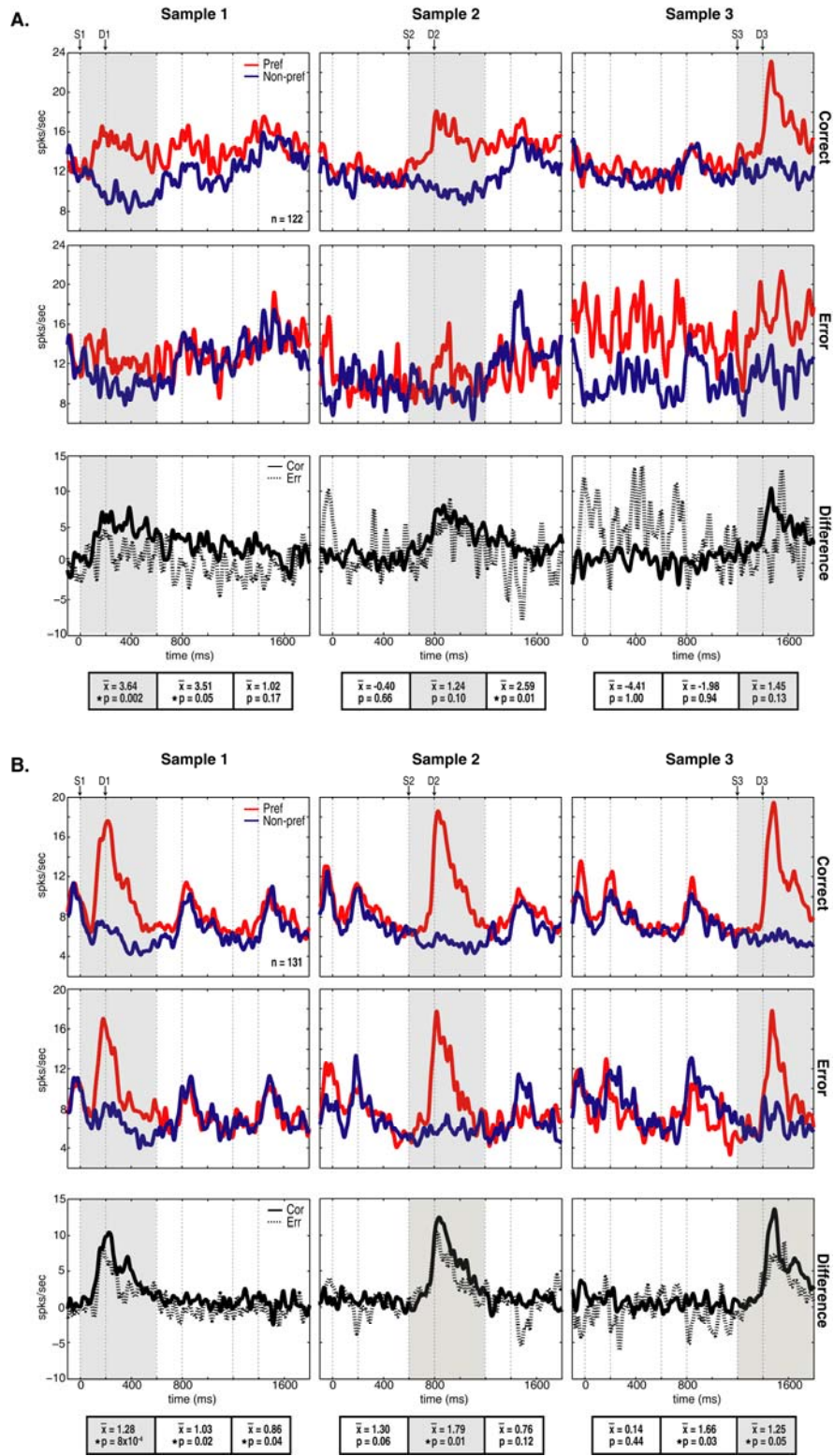


Figure 20. Discriminative signal differences between correct and error trials during the Spatial Sternberg task.

Figure 20. (continued)

Data from Monkeys 1 and 2 are shown in A and B (respectively). Preference was determined based on activity within the shaded region. Top rows: correct trial PSTHs; Middle: error PSTHs; Bottom: Correct and error trial discriminative signals. Boxes: \bar{x} = difference between correct and error mean discriminative signals. P values of the two-tailed paired t-test are given below. * indicate significant p values (<0.05).

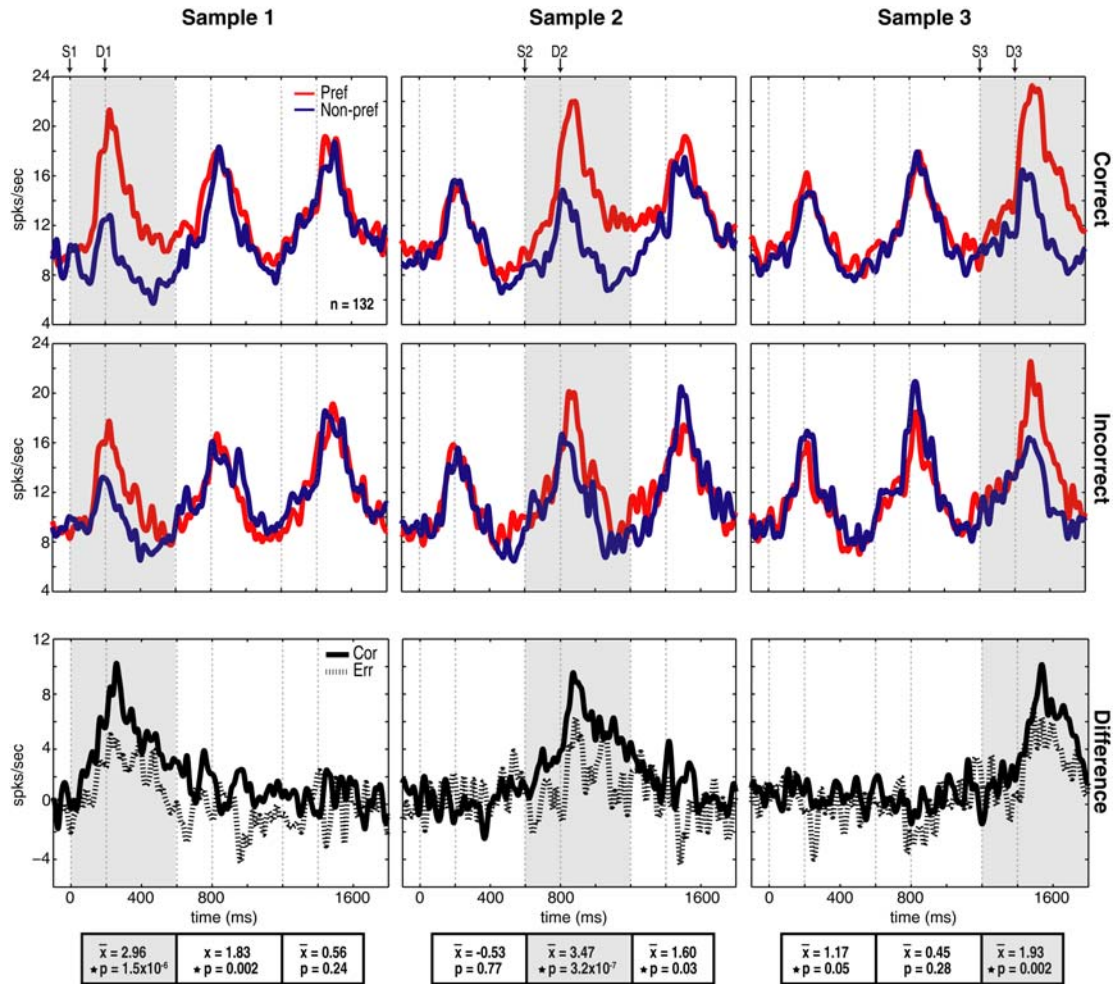


Figure 21. Discriminative signal differences between correct and error trials during the Color Sternberg task.

Monkey 1 only. Preference was determined based on activity within the shaded region. Top rows: correct trial PSTHs; Middle: error PSTHs; Bottom: Correct and error trial discriminative signals. Boxes: \bar{x} = difference between correct and error mean discriminative signals. P values of the two-tailed paired t-test are given below. * indicate significant p values (<0.05).

5.3.2 Selectivity on fast vs. slow trials

Selectivity for sample identity was evident on both fast and slow trials and generally did not persist into subsequent epochs. Results from the two-tailed paired t-tests showed that there was a tendency for the depth of selectivity to be greater on fast trials. Significant differences were mostly restricted to the current epoch. Differences in discriminative signal between fast and slow trials was much less robust than those observed between correct and error trials (Figures 22 and 23).

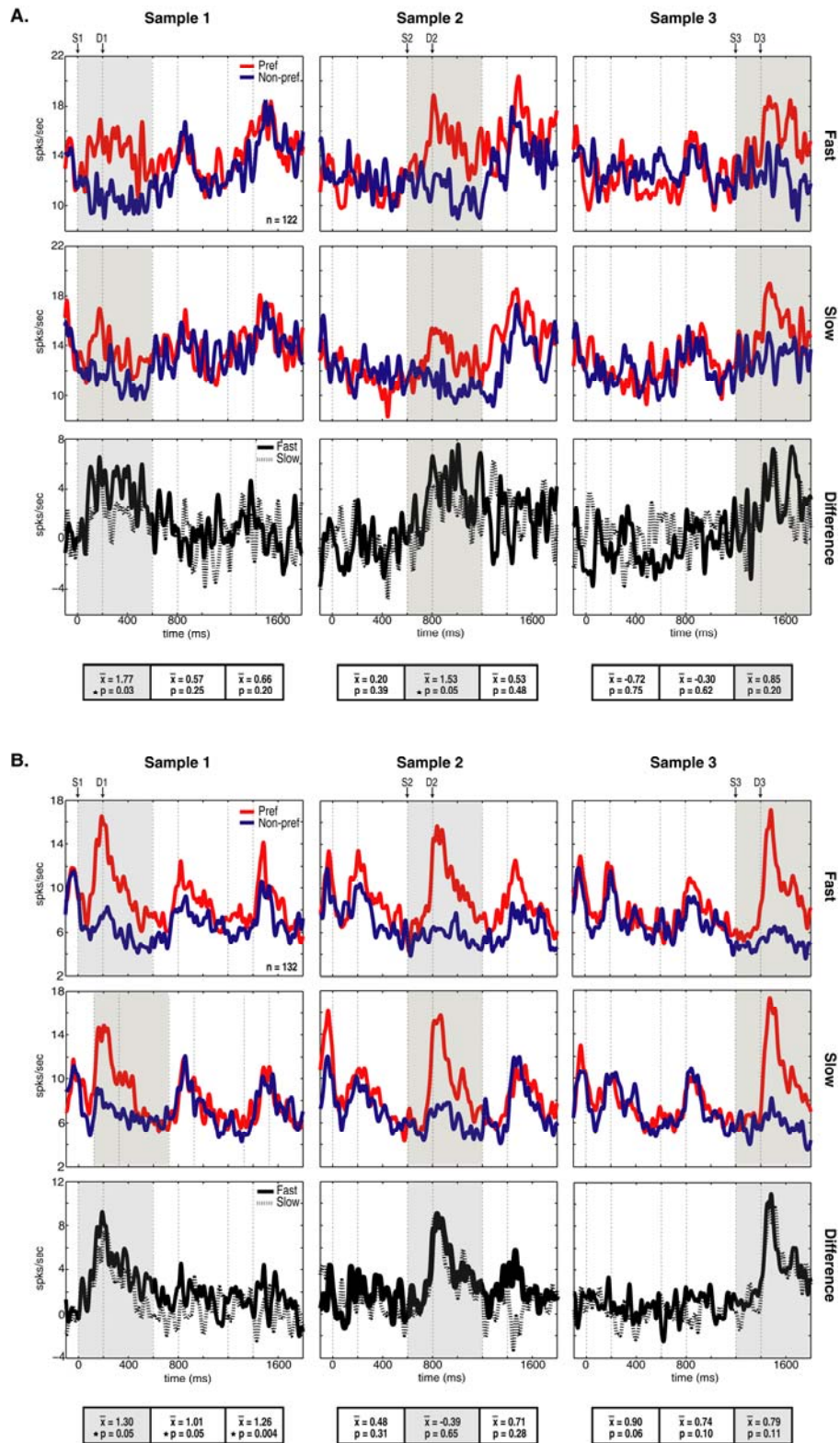


Figure 22. Discriminative signal differences between fast and slow RT trials during the Spatial Sternberg task.

Figure 22. (continued)

Data from Monkeys 1 and 2 are shown in A. and B. (respectively). Preference was determined based on activity within the shaded region. Top rows: fast RT trial PSTHs; Middle: slow RT PSTHs; Bottom: Fast and slow trial discriminative signals. Boxes: \bar{x} = difference between fast and slow mean discriminative signals. P values of the two-tailed paired t-test are given below. * indicate significant p values (<0.05).

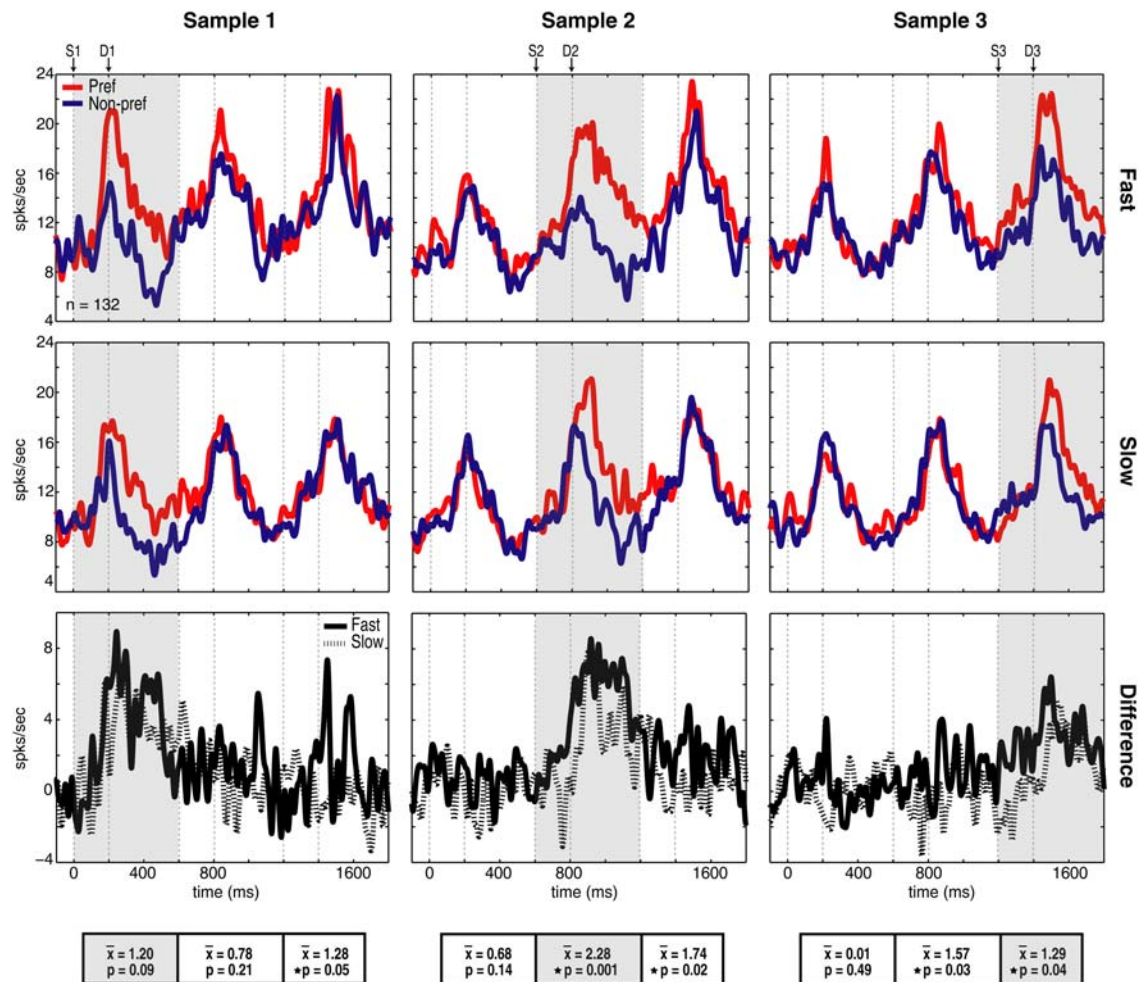


Figure 23. Discriminative signal differences between fast and slow RT trials during the Color Sternberg task.

Data from Monkeys 1 and 2 are shown in A. and B. (respectively). Preference was determined based on activity within the shaded region. Top rows: fast RT trial PSTHs; Middle: slow RT PSTHs; Bottom: Fast and slow trial discriminative signals. Boxes: \bar{x} = difference between fast and slow mean discriminative signals. P values of the two-tailed paired t-test are given below. * indicate significant p values (<0.05).

5.4 DISCUSSION

Previously, we demonstrated that on correct trials the majority of PFC neurons were significantly selective for sample identity during the delay-period of the Sternberg working memory task, but was this signal relevant for performance? Decreased delay activity on error trials and trials when the monkey made slower responses suggest that it is.

On trials when performance was optimal (fast and correct) we observed that the depth of selectivity during the delay was stronger when compared to trials on which performance was not optimal (slow and incorrect). It had been shown that electrically stimulating PFC during the delay resulted in performance deficits (Sobotka et al., 2005). Decreases in selectivity accompanied the performance deficits. The authors concluded that performance errors were the result of weakened item representations in memory. Our results are consistent with these and similar findings that have made this claim (Funahashi et al., 1989; Fuster, 1973, Inoue and Mikami, 2006; Sawaguchi and Yamane, 1999). However, these differences were largely restricted to the current epoch making it difficult to interpret how these signals may be used for response selection. The absence of a sustained response in PFC during a working memory task has also been observed in human fMRI experiments (Postle et al., 2003). In these experiments, humans performed a delayed match to sample task using faces. The number of items in a trial was varied from 2-4. The authors reported that PFC delay-period activity was not sustained throughout the intervening delays of the trial. They did however observe sustained activity in the fusiform face area. Based on this finding the authors concluded that memoranda for items are

stored in a modality specific manner. It is possible that PFC does not store the representations of items in working memory. This could explain why the effects of performance on selectivity observed in the current study were restricted to the delay that immediately followed the sample.

It is also possible that sustained delay-period activity is not the mechanism for maintenance. In monkey extrastriate visual cortex, Lee et al. (2005) observed that theta oscillations were closely coupled to the timing of single unit spike discharges during the delay of the delayed match-to-sample task. It was observed that single unit activity varied systematically with the angle of LFP theta oscillations. Because of these systematic variations with the theta angle it was possible to define a preferred angle for each neuron. The authors found that sample selective activity occurred only when spikes fired at the preferred angle. The alignment of single unit activity to theta oscillations and its associated effects on stimulus selectivity occurred largely in the absence of sustained increases in the mean firing rate of neurons during the delay-period. It is not known if this occurs in PFC, however this experiment describes a mechanism by which neurons can represent stimulus identity in the absence of a sustained increase in firing rate during the delay.

Though we did not observe sustained selectivity in PFC, none-the-less this activity significantly correlated with performance. Maintenance could be supported by another mechanism other than sustained activity (such as phase-locking to the theta rhythm) or by a memory circuit involving PFC and other brain areas that store the sensory specific information. This possibility has yet to be fully described.

6.0 GAMMA SYNCHRONIZATION IS NOT MODULATED BY WORKING MEMORY LOAD

6.1 INTRODUCTION

Cortical oscillations can vary both in their underlying neural mechanisms as well as in the types of perceptual or cognitive events that elicited them. They can arise from chemical or electrical synaptic interactions within networks of neurons, or from ‘pacemaker’ neurons due to their intrinsic membrane properties (for reviews, see Jefferys, Traub et al. 1996; LeBeau, Traub et al. 2003; Wang, 2003). Oscillatory activity can be driven exogenously and is therefore phase-locked to the stimulus (evoked) or may be driven by endogenous processes which are out of phase with the stimulus (induced) (Galambos, 1992). The cognitive mechanisms underlying evoked and induced responses are different for instance: evoked gamma (30-80 Hz) responses are thought to possibly mediate perceptual binding whereas induced gamma responses are thought to be associated with higher cognitive processes (see Tallon-Baudry 1999 for review).

There is growing evidence that synchronization of cortical neuronal activity in the gamma band is associated with various types of information processing. Studies have demonstrated that visual stimuli can elicit gamma band synchrony in the cat (Eckhorn, Bauer et

al. 1988; Gray and Singer 1989) and monkey (Kreiter 1992; Frien, Eckhorn et al. 1994) visual areas with analogous findings in human EEG studies (Lutzenberger, Pulvermuller et al. 1995; Muller, Bosch et al. 1996). Findings of gamma band synchrony span various spatial scales ranging from intra- (Gray and Singer 1989) and inter-areal (Frien, Eckhorn et al. 1994; Roelfsema, Engel et al. 1997) to interhemispheric (Engel, Konig et al. 1991) sets of neurons. Synchronous gamma band activity has been proposed to be critical for perceptual feature binding (Von der Malsburg 1983; Singer and Gray 1995; Tallon-Baudry 1999); but see (Shadlen and Movshon 1999) for critique of this hypothesis). Studies have reported that gamma band synchrony is not only associated with motor and sensory processing but also extends to higher cognitive processes like working memory (Tallon-Baudry, Bertrand et al. 1998; Tallon-Baudry, Bertrand et al. 1999; Howard, Rizzuto et al. 2003). This suggests that gamma synchrony may be a more generalized mechanism for entraining networks of cortical neurons in the service of forming and maintaining representations.

Working memory allows us to keep items “in mind” after they are no longer available to us as physical stimuli. Keeping items in mind requires that representations of these items be sustained until they are used for some subsequent action. The amount of information that must be held in mind is referred to as working memory load. Howard et al. (2003) reported that gamma oscillations in human subjects increased linearly with increasing load. This study provided the first evidence that gamma oscillations support multi-item working memory. Specifically, it has been suggested that gamma oscillations support the organization and temporal segmentation of multiple items in working memory (Lisman and Idiart, 1995; Luck and Vogel, 1997; Jensen and Lisman, 1998).

In the present study, we examined whether increased working memory demands, manifested by higher working memory load, modulate induced gamma band synchrony in monkey prefrontal cortex. We measured local field potential (LFP) activity in two monkeys while they performed a version of the Sternberg working memory task that varied demands for working memory load between trials. In the low-load condition, the monkey only had to maintain one item in memory. In contrast, the high-load condition where the monkey had to remember three items placed much greater demands on working memory as evidenced by behavioral performance. If gamma band activity is indeed an electrophysiological signature of working memory load, then we would expect increased gamma band synchronization during the delay-period for high working memory load trials.

6.2 METHODS

6.2.1 Subjects and neuronal recordings

Two adult male monkeys (*Maccaca mulatta*) were surgically prepared for neuronal recordings in the right (Monkey 1) and left (Monkey 2) prefrontal cortex, as described previously in Chapter 3. All procedures and experiments were conducted under the supervision of the University of Pittsburgh Institutional Animal Care and Use Committee and complied with the guidelines set forth in the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

Data collection procedures were identical to those detailed previously (Chapter 3). Briefly, PFC was identified using structural MRI to guide the placement of the recording chamber. The recording chamber accommodated a plastic plug containing vertical holes spaced 1 mm apart so as to form a square grid (Crist et al., 1988). The grid was inserted and fixed in place with set screws during each recording session. A tungsten electrode with an initial impedance of $\sim 5.0\text{ M}\Omega$ at 1 kHz (Frederick Haer Co.) was advanced through the guide tube using a Narashige hydraulic microdrive affixed to the chamber. The neural responses were monitored at 129 recording sites (approximately 43 sites per task for each monkey). The local field potentials (LFP) were sampled at 1000 Hz and bandpass filtered 0.7-170 Hz. The band-pass filtered traces from these sites formed the LFP database. A computer ran Plexon (Plexon Inc.) software for on-line LFP monitoring. The times of the action potentials and other events were sampled at 40 kHz. Data from all trials were displayed on-line and saved to disk. Signals from the electrode were led to the Plexon system, analogue and digital oscilloscopes, and an audio monitor.

6.2.2 Behavioral task

Monkeys performed the Sternberg cued-recall task described in Chapter 2 (Figure 1.). Monkey 1 was trained on two different versions of the task; one using color stimuli the other using spatial stimuli. Monkey 2 adequately learned the spatial version of the task only. Performance on the task was significantly above chance (33.3%) for both monkeys. Monkey 1 was 86% correct on the spatial task and 69 % correct on the color the task. Monkey 1 had a mean reaction time of

202 ms on the spatial and 250 ms on the color version. Monkey 2 was 70 % correct and had a mean reaction time of 252 ms.

6.2.3 Data Analysis

Off-line processing

Data were filtered off-line using a 60 Hz notch filter. Epochs were defined as -400 to 2100 ms relative to sample one onset. We extended the epochs beyond the analysis epoch to avoid introducing edge effects from the time-frequency transformation of the data. Error trials were excluded from analysis.

Time-frequency transformation of the data

Time-frequency analyses were carried out using Matlab (*MATLAB*© version 7.4.1, 2007, The MathWorks Inc., Natick, Massachusetts). The wavelet transformation was applied using the complex Morlet wavelet defined by:

$$mo(x) = c \cdot e^{-\frac{x^2}{2}} \cdot e^{i\omega_0 x},$$

which describes a family of functions that oscillates according to the frequency parameter ω_0 with a Gaussian envelope $\exp(-x^2/2)$ and a factor $c = (\sigma\sqrt{\pi})^{-1/2}$ that appropriately normalizes the total energy to 1.

Wavelet analyses decomposed the signal between 15 and 125 Hz (110 frequency steps) into its time-frequency components. Induced responses are not time-locked to the stimulus and are determined by averaging the segments after they have been wavelet transformed. Upon inspection of the wavelet spectrogram for the entire frequency range we found that the task related signal for the gamma band range was centered around 90 Hz. For further analyses we defined the gamma band signal to be the average power values from 80-100 Hz. Contrasts of the high- vs. low-working memory load were carried out by comparing gamma power in the first delay to gamma in the third delay on both the experimental and control trials. Comparisons were performed using the non-parametric Wilcoxon signed rank test for matched pairs across sessions for each monkey. Statistical analyses were carried out over a 100 ms moving window shifted by 50 ms. We accounted for multiple comparisons using Bonferroni correction of the p values.

6.3 RESULTS

6.3.1 Gamma band synchrony does not increase with working memory load

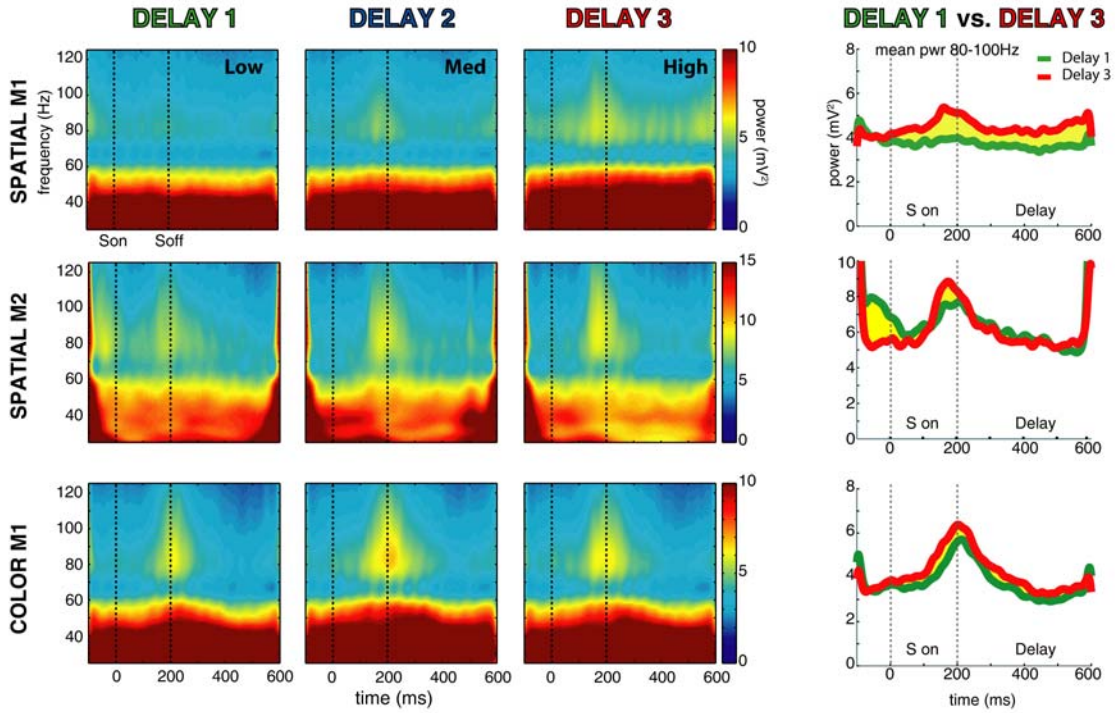
We found that gamma band activity increased during each of the three delay-periods. The gamma band responses were not sustained across the trial and were attenuated with the presentation of the next sample. Monkey 1 exhibited successive increases in gamma power from the first delay to the third delay. In the experimental condition, gamma power in the third delay-period was significantly greater than in the first delay-period (Wilcoxon, $p < 0.0043$) (Figure

24A). These effects were also observed on the control trials where load remained constant (Figure 24B). In Monkey 2, delay-period gamma band activity was overall higher during the course of the trial when compared to baseline. However, unlike Monkey, gamma band responses in Monkey 2 did not increase from the first to the final delay-period on either the experimental or control trials.

6.3.2 Firing rate does not increase with working memory load

Increases were also observed in the mean firing rate of single units as samples were presented successively (Figure 25). These firing rate increases paralleled the increases that were observed in gamma band power. Monkey 1 exhibited higher firing rates on control trials when compared with the experimental trials. Monkey 2 exhibited no significant change in the firing rate across the three delay-periods in either condition.

A. Experimental Condition



B. Control Condition

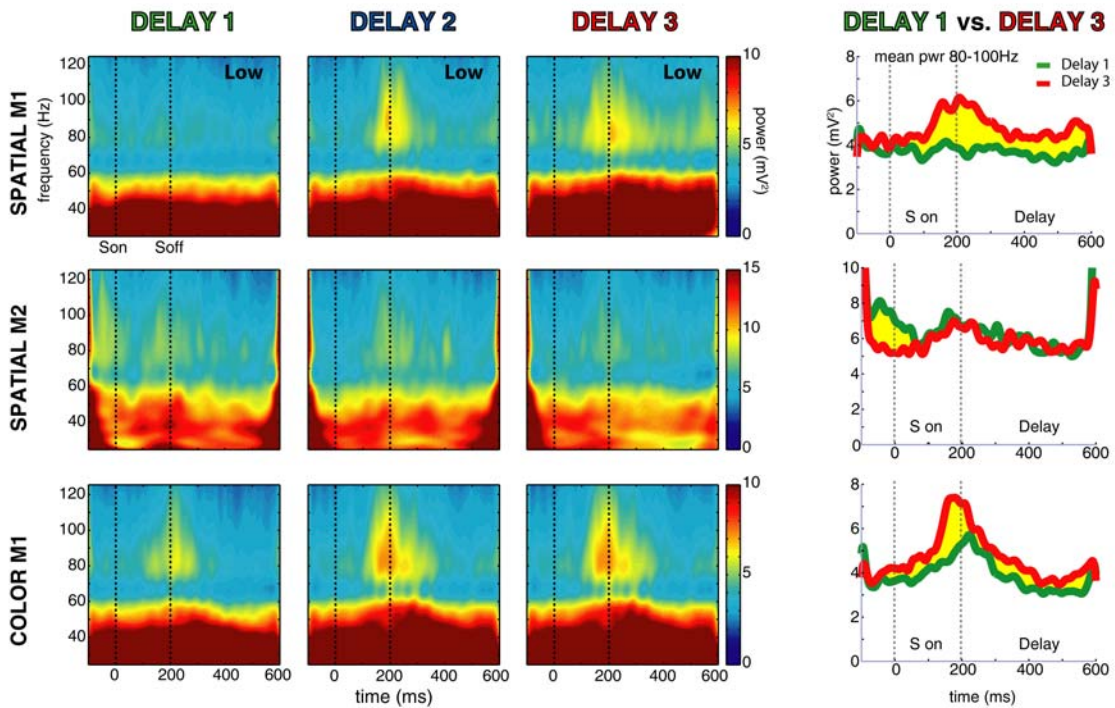


Figure 24. Gamma band activity with increasing memory load.

Figure 24. (continued).

A. Experimental condition: working memory load increases from low (1 item) to high (3 items) across delay-periods 1 thru 3. B. Control Condition: working memory load remains low (1 item) across delay-periods 1 thru 3. Left: average spectrogram of power (25-125 Hz) for each monkey across delays. Right: delay 1 vs. delay 3 mean power from 80-100 Hz. Shaded area identifies periods of significant difference in gamma power between delays 1 and 3.

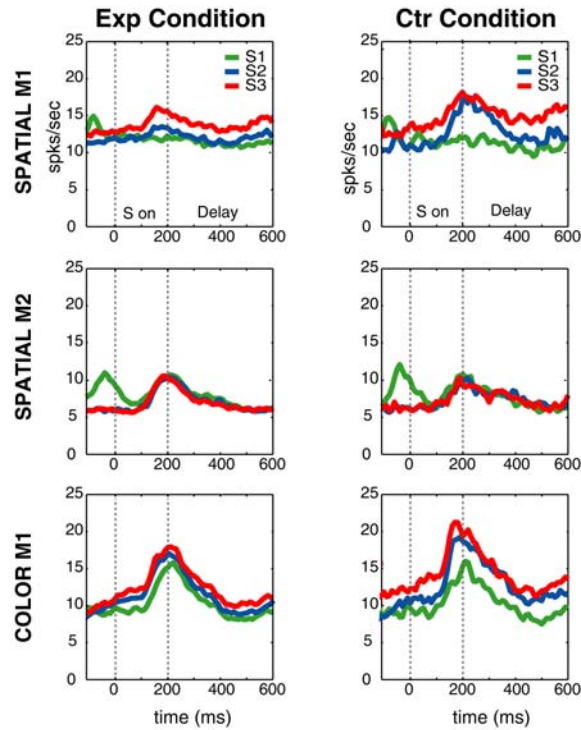


Figure 25. Firing rate across delay-periods 1 thru 3.

Average PSTH's aligned on samples 1, 2, and 3 are shown in green, blue, and red respectively. Monkey 1 (top and bottom panels) showed increased firing rates as the trial progressed regardless of working memory load demands (compare experimental to control). Monkey 2 (middle panel) firing rate differences across epochs did not vary greatly.

6.4 DISCUSSION

We measured gamma band activity across delay-periods as working memory load was incrementally increased from one to three items. We found that Monkey 1 exhibited both increased firing rate and power in the gamma frequency band of the LFP as samples were presented successively; however these effects were also observed in the control condition where load remained constant. Monkey 2 exhibited no significant change in firing rate or in gamma power across epochs. Contrary to what has been observed in humans, our data reveal that gamma power does not correlate with working memory load in monkeys.

What can account for this discrepancy? It may be that monkey working memory is not supported by the same neural mechanisms that support human working memory. This would be unlikely since it has been observed that performance on such tasks is very similar between humans and monkeys (Sands and Wright, 1980; Wright, 1985). It is difficult to imagine that such similar behaviors could arise from very different underlying mechanisms. However, this possibility cannot be completely ruled out.

Another possibility is that increases in gamma responses reported previously were not due to increases in working memory load but some other factor that was correlated with load in the task. Gamma may be a correlate of the passage of time or of other factors that may modulate throughout the course of the trial such as attention and/or arousal. Unlike the present study, the task employed in the previous experiments did not include trials that would have controlled for these factors; therefore the possibility cannot be ruled out.

Though changes in gamma band activity were not modulated by working memory load increases, they were evident in the delay-period activity of the LFP signal across the trial. It is correlated neuronal activity in PFC could explain the observed increases in delay-period gamma power. This has been demonstrated to be the case in human auditory cortex (Nit et al., 2007). Increases in gamma power were found to be tightly coupled to increases in correlated activity distributed across the neuronal population. These increases in spike-gamma coupling were not due to evoked responses by stimuli because these increases were found to occur during spontaneous activity as well. Spike-gamma coupling also could not be explained solely by increases in firing rate. When selecting trials that had equal average firing rates, gamma power increases were only observed when accompanied by increases in correlated neuronal activity.

In conclusion, gamma power and firing rates were found to increase during the delay-period. However these changes could not be attributed to increases in working memory load. It is possible that increases in correlated activity distributed across the neuronal population underlie the observed gamma power increases. Factors such as attention and/or arousal that modulate throughout the course of the trial could contribute to increases in correlated spike activity and gamma band synchrony.

7.0 GENERAL DISCUSSION

A vastly accumulating body of research strongly suggests that the prefrontal cortex (PFC) is critical in supporting working memory function. Human (for review see Baldo and Shimamura, 2002) and monkey (Funahashi et al, 1993; Fuster, 1989; Goldman-Rakic, 1987; Jacobsen, 1936; Sawaguchi and Goldman-Rakic, 1991) lesion studies have demonstrated that damage to prefrontal areas results in working memory impairment. In studies where PFC is intact, it has been reported that PFC activity changes when engaged in working memory tasks (for review Miller and Cohen, 2001; Tanji and Hoshi, 2008).

Much of what we know about working memory-related neuronal changes comes from monkey neurophysiological studies. It has been reported that activity increases during the delay when monkeys are required to maintain a representation of a previously displayed stimulus during delayed response tasks (Funahashi et al, 1989; Funahashi et al, 1993; Fuster and Alexander, 1971; Miller et al, 1996). Furthermore, this delay-period activity is selective for the items held in memory (Fuster and Alexander, 1971; Kojima and Goldman-Rakic, 1982; Kubota and Niki, 1971; Funahashi et al, 1989), a necessary feature of an area subserving working memory function. In addition to exhibiting selectivity, delay-period activity in PFC has also been shown to have behavioral relevance. Many studies have demonstrated that the strength of delay period activity is correlated with performance (Funahashi et al., 1989; Fuster, 1973, Inoue and Mikami, 2006; Pessoa et al., 2002; Sawaguchi and Yamane, 1999).

Changes at the population level have also been observed in humans using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). Studies have demonstrated that prefrontal regions are selectively activated in tasks utilizing working memory (Fiez et al., 1996; Jonides et al., 1993; Petrides et al., 1993). There is evidence that PFC gamma power also increases during the delay while subjects performed the Sternberg task (Howard et al., 2003).

Neurophysiological experiments in both humans and monkeys have demonstrated the existence of selective sustained delay-period activity in PFC. It is a strongly held belief that this activity is the neural signature of working memory. Working memory is one of our most crucial cognitive abilities. There have been many neurophysiological studies that examine how single items are maintained in the activity of prefrontal neurons. However little is known about the encoding of multiple items. This is an important question because complex behaviors require that many items be held in memory at one time.

There is a relative paucity of experiments examining the activity of prefrontal neurons in the context of a multiple item working memory task as this has only recently begun to be explored (Inoue and Mikami, 2006; Warden and Miller, 2007 and 2010). Data published to date are at times discordant; however there are a few similarities between them. Though the information reported to be carried in the delay-period activity varied between these studies (Inoue and Mikami, 2006; Warden and Miller, 2007 and 2010), they all reported that the identity of a single item represented in the delay-period activity of PFC neurons becomes attenuated as new items are added to memory.

The overarching goal of this dissertation was to examine how the requirement to hold multiple items in memory affects performance and neuronal activity in monkey prefrontal cortex.

We found that monkeys were able to perform a task requiring the memory for multiple items and that the monkeys' performance reliably exhibited effects of both working memory load and recency. Our data are consistent with findings reported by Buschman et al. (2011) who examined load effects on performance during a working memory task in monkeys. In their study, working memory load was manipulated independently in each hemifield of space. The authors reported that the monkey's performance fell significantly with larger sets of items within the hemifield.

We found that delay-period activity, both firing rates and gamma synchrony, weakened as new items were added to memory. The memory for items, represented by selective delay-period activity, was largely restricted to the delay that immediately followed these items. That is to say, PFC activity during the delay most strongly represented information of the most recently presented item. Our data as well as data from previous studies (Inoue and Mikami, 2006; Warden and Miller, 2007 and 2010) have shown that information for items held in memory was significantly degraded by the end of the trial.

The attenuation of sustained selective responses is not something unique to multi-item tasks. Other studies using single items have also reported weak and/or transient item selectivity during the delay (Meyers et al., 2012; Zaksas and Pasternak, 2006). Work done by Meyers et al. reported that the accuracy of predicting object identity was strongest during the presentation of the sample and dropped considerably during the delay. Item identity was not strongly represented during the delay; however this stood in stark contrast to the strength of representations for the task rule which was very strongly represented during the delay, suggesting that PFC may be a less important structure for representing stimulus properties but highly important for goal and state representations.

The results from these and other experiments present us with the conundrum that although monkeys were capable of performing a task requiring the memory for multiple items, delay-period activity in prefrontal neurons cannot easily explain the monkey's working memory performance. How can we reconcile these findings?

7.1 MEMORY TRACE EXISTS IN ANOTHER REGION OF PFC

One possible explanation for the lack of a sustained working memory signal for items could be that the signal exists elsewhere in prefrontal cortex. Given the extensive literature on working memory, peri-principal prefrontal cortex was a logical candidate as the site of storage for multiple items held in working memory. This region has been described as having selective working memory delay-period activity during delayed response tasks (Fuster and Alexander, 1971; Kojima and Goldman-Rakic, 1982; Kubota and Niki, 1971; Funahashi et al., 1989), a necessary feature of an area subserving working memory function. The delay-period activity in lateral PFC has also been shown to have behavioral relevance. Several studies have demonstrated that the strength of delay-period activity is correlated with performance (Funahashi et al., 1989; Fuster, 1973; Inoue and Mikami, 2006; Pessoa et al., 2002; Sawaguchi and Yamane, 1999). Lesions to peri-principal PFC have been shown to produce deficits selective to memory-guided saccades while sparing saccades made to a visible cue (Funahashi et al., 1993; Sawaguchi and Goldman-Rakic, 1991). Though activity in the lateral PFC has been the focus of most working memory research, other frontal areas have been found to demonstrate working memory-related activity. These areas include: the inferior convexity (Romo et al., 1999; Brody et al., 2003), premotor area 6 (e.g., Postle, Stern, Rosen, & Corkin, 2000; Awh et al., 1996; Baker,

Frith, Frackowiak, & Dolan, 1996; Jonides et al., 1993) and superior frontal areas 6 and 8 (e.g., Postle & D'Esposito, 1999; Mellet et al., 1996; Smith, Jonides, & Koeppe, 1996; Sweeney et al., 1996). Though the evidence in support of lateral PFC working memory function is strong it cannot be ruled out that working memory may be mediated by other areas in prefrontal cortex.

7.2 MEMORY TRACE EXISTS OUTSIDE PREFRONTAL CORTEX

It is possible that the representation of multiple items is not maintained in PFC, but in other regions of the brain. Delay-period activity is not exclusive to PFC. In other areas of the brain studies have reported a delay-period maintenance signal similar to that which has been described in PFC. Sustained delay-period activity has been reported in both human and monkey prefrontal, inferotemporal, and parietal cortices (Andersen and Buneo, 2002; Chaffee and Goldman-Rakic, 1998; Freedman et al., 2003; Goldberg et al., 2002; Curtis and D'Esposito, 2003; Glimcher, 2003; Passingham and Sakai, 2004; Zaksas and Pasternak, 2006). Observations from intracranial EEG experiments in humans found modulations of theta synchrony by working memory load in occipital, temporal, and parietal cortices. Moreover, these modulations were absent in frontal regions of the brain. Other studies have also reported similar modulations of BOLD and ERP signals in humans restricted to posterior regions of the brain (Ikkai et al, 2010; Vogel and Machizawa, 2004; Postle et al., 2003) suggesting that the delay-period activity in these areas may play an important role in working memory function.

In support of this hypothesis, extensive review of several human lesion studies, D'Esposito and Postle (1999) found that patients with parietal lobe lesions displayed markedly worse working memory performance compared to prefrontal lesioned patients or normal

controls. In fact, they found that patients with prefrontal lesions were generally not impaired on span and delayed response tasks. In those who did show impairment, deficits were most prominent under conditions of distraction during the delay-period. Evidence from monkey lesion studies is consistent with these observations. Funahashi et al (1993) reported that after PFC ablation postoperative memory-guided saccades occurring after 1.5 sec delay-periods remained largely intact, therefore it seems that responses selected after shorter delays were guided by information stored elsewhere in the brain. Postoperative responses following delays extending beyond 1.5 sec were less accurate though generally they were directed towards the correct location. Wajima and Sawaguchi found that after administration of the GABA_A antagonist bicuculline, performance on oculomotor delay response task (ODR) was reduced but not abolished. Consistent with previous findings, they reported that monkeys were less accurate after injection. However, upon closer inspection of the error trials, they found that the selected target usually corresponded to the correct response for the preceding trial. Therefore, it did not appear that disrupting activity in PFC abolished the memory trace, but affected the retrieval of this information for the use of response selection.

Combined these data suggest an alternative hypothesis that working memory storage is subserved by domain specific areas in the brain, and the role of PFC supports the executive control component of working memory by means of maintaining task-set and manipulating or altering representations to organize behavior.

7.3 SUSTAINED FIRING RATES DO NOT SUPPORT WORKING MEMORY

The absence of sustained delay-period activity across the trial may give us cause to reconsider the idea that the working memory trace is maintained by a sustained rate code of neuronal firing and to consider instead an alternative mechanism. One possibility is that maintenance is achieved via a form of temporal coding. Computational modeling and LFP analysis lend support to the idea that neuronal firing that is temporally irregular can still carry information during a memory delay (Pesaran et al., 2002; Lee et al., 2005). Lee et al. examined the activity in monkey V4 while subjects performed a delayed match-to-sample task. They observed that the mean firing rate did not change significantly between the fixation and the delay periods. Based on one-factor ANOVA analyses of delay period firing rate, they found that only 17% of the neurons were significantly modulated by the identity of the sample. Upon further inspection of the data, the authors observed that theta oscillations were closely coupled to the timing of action potentials of single neurons during the delay-period in a manner where single unit activity varied systematically with the angle of the LFP theta oscillation. These systematic oscillations were used to define a preferred theta angle for each neuron. Taking into account the angle of the LFP theta oscillation significantly increased the estimate of how many V4 neurons contributed to working memory to 58%. The authors found that during the delayed match-to-sample task, encoding of the identity of the remembered stimulus occurred near this preferred theta angle. Both the alignment of single unit activity to theta oscillations and the effects of this alignment on stimulus selectivity occurred largely in the absence of overall increases in the mean firing rate of neurons during the delay-period.

Another possible mechanism for maintenance is that the representations of remembered items arise via short-term changes at the level of the synapse. Brief activity in a synapse can

enhance or diminish its subsequent strength. It is possible that the transient responses during the delay-period are sufficient to alter these strengths within a network of neurons on a trial-by-trial basis. These dynamic weight changes could serve to alter what information is represented by the network within a given trial.

7.4 MEMORY ENCODED IN PFC IS DISCRETE AND DYNAMIC

Experimental approaches investigating the averaged activity of population of neurons make the assumption that the population is largely homogenous and that these neurons perform similar functions over a similar time course. More recent data suggest that the activity of neurons within PFC may reflect more fractionated functions and that the contributions that they make to elicit a behavioral response may not be unitary (Inoue and Mikami, 2006). That is to say that separate populations of neurons make specialized contributions at different times and that task-relevant information is passed across different functional units across a trial. Data that support this claim have revealed that information during the delay is supported by small distributed populations of neurons in PFC. Encoding of information within these populations occurs over very small temporally discrete intervals. It has been demonstrated that the information carried by these populations changes rapidly and is continually passed from one subset to another over the course of a trial. Our data encoded over considerably larger intervals and would not have captures such discrete temporal dynamics.

7.5 MEMORY TRACE IS ENCODED IN A NON-LINEAR MANNER

A final possibility we consider is that delay-period firing rates do carry item information however the code is non-linear and cryptic. That is to say, that the code carried in delay period activity when more than when item is in memory is more complicated than some linear transformation of the activity elicited by individual objects. It is difficult to imagine that an area with relatively easily defined selectivity during the delay for single items would adopt such a code for multiple items, however this hypothesis is supported by findings reported by Warden and Miller (2007). They found that adding a second item to memory changed the activity related to the first item in a non-systematic fashion. Delay-period selectivity for two items could not be predicted by the neuron's response to items presented alone. This possibility would not be revealed with the analyses employed in the current study which were based on the assumption that the code is a straightforward one which reflects either a linear main effect of the sample item or a non-linear interaction of stimulus combinations. There is little evidence to support the idea of a more complex code and would require more extensive research.

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